Manual of Phytoplankton Primary Production Methodology

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ABSTRACT

Shearer, J.A., E.R. DeBruyn, D.R. DeClercq, D.W. Schindler, and E.J. Fee. 1985. Manual of phytoplankton primary production methodology. Can. Tech. Rep. Fish. Aquat. Sci. 1341: iv + 58 p.

Procedures are described for the estimation of integral phytoplankton production using an artificial light incubator and a numerical model. The method requires measurement of three key functions: surface irradiance versus time, light attenuation versus depth and phytoplankton production versus irradiance. Field and laboratory procedures are detailed and examples are given. Rationale for the use of specific techniques is also included.

Key words: primary production; methodology; phytoplankton; planktonology; photosynthesis; incubation; biological sampling; light measurement; light attenuation; modelling; mathematical models; computer programmes.

RESUME

Shearer, J.A., E.R. DeBruyn, D.R. DeClercq, D.W. Schindler, and E.J. Fee. 1985. Manual of phytoplankton primary production methodology. Can. Tech. Rep. Fish. Aquat. Sci. 1341: iv + 58 p.

Ce rapport décrit les méthodes utilisées pour évaluer la production globale de phytoplanctons à l'aide d'un incubateur artificiel à lampe et d'un modèle numérique. Dans cette méthode, on doit tenir compte de trois facteurs-clés: l'irradiation de surface en fonction du temps, l'atténuation de la lumière en fonction de la profondeur et la production de phytoplanctons en fonction de l'irradiation. Ce rapport décrit les méthodes utilisées en laboratoire et sur le terrain et en donne des exemples. On y justifie aussi l'emploi de certaines techniques.

Mots-clés: production primaire; méthodologie; phytoplancton; planctonologie; photosynthèse; incubation; échantillonnage biologique; mesure de la lumière; atténuation de la lumière; établissement de modèles; modèles mathématiques; programmes informatiques.

I. GENERAL INTRODUCTION

A. BACKGROUND

In 1968, when we began studies at the Experimental Lakes Area (E.L.A) northwestern Ontario, we tested both the O_2 and ^{14}C methods of measuring primary production (Schindler and Holmgren 1971). Neither method was adequate for our purposes. The former was too insensitive for use in oligotrophic lakes. The latter required measurements of the dissolved inorganic carbon (DIC) concentration, but methods at that time did not have sufficient sensitivity. This problem was overcome by the development of rapid, sensitive methods for DIC determination by gas chromatography (Stainton 1973; Stainton et al. 1977), by conductimetric techniques (Stainton et al. 1977), or by infra-red analysis (Section II.B.1).

A second problem with the ^{14}C technique was the correction for filtration error, suggested by Arthur and Rigler (1967). This correction proved to be an artifact, caused by an inverse correlation between the size of sample filtered and the proportion of ^{14}C-labelled colloidal material retained by a filter. The colloid fraction was found to be labelled as part of the photosynthetic process rather than by adsorption, so it validly should be included in the assay of "produced" material. The need for this correction was eliminated by replacing filtration with an acidification and bubbling procedure to drive off unutilized DI^{14}C after incubation (Schindler et al. 1972), followed by radioassay of the remaining activity, in dissolved plus suspended organic matter, with liquid scintillation methods (Schindler 1966).

In situ incubation was found to yield results which were difficult to compare between lakes, due to the unpredictable nature of the light regime in response to weather changes. By substituting an incubator where samples could be incubated in a preset, quantifiable light gradient, changes in the photosynthesis-light response could be measured. From these, production in the lake could be predicted accurately for any time period, if accurate light data were collected (Fee 1973a, 1973b). In addition, the incubator made it possible to compare photosynthesis-light responses for several lakes under identical light regimes. The incubator has been tested and refined over the years into a simple, reliable device which can be produced inexpensively and maintained easily, even in remote areas (Shearer 1976; Appendix I).

Consequently, the current method for measuring phytoplankton production at E.L.A. bears little resemblance to standard in situ techniques (Vollenweider 1974). We have tested the critical components of the methods and we are confident of the results. We have had an increasing demand for description and documentation of the technique. This manual is designed to summarize several references and years of development of the E.L.A. incubator method and to explain the current procedures for estimating phytoplankton production. We continue to refine and simplify these procedures and hope to update this manual periodically.

B. THE MODEL

The methods described in this manual allow one to acquire data for a numerical model for the estimation of integral phytoplankton production. Some

aspects of this methodology are specific to the model being used, but most have a much broader application.

The basic empirical model was developed by Fee (1973b) and refined over the years. It requires, as input, three types of measured data. These are:

i) the flux of surface solar irradiance over time (irradiance vs. time). Measurement of this relationship is discussed in Section II.D.
ii) the vertical attenuation of solar irradiance with depth in the water column (irradiance vs depth). This measurement is described in Section II.C.
iii) the photosynthetic response of phytoplankton to a gradient of irradiances (production vs irradiance). This function can be determined in situ. However, we prefer to use an artificial light incubator (see Section II.B) for reasons outlined earlier and discussed in detail later.

Of all these input parameters, the common factor is irradiance. This allows the model to estimate photosynthesis at any depth over any time period, based on the surface solar irradiance measured for that time period. Linear interpolation is used to estimate values for times and depths falling between those actually measured.

Figure I.B.1 provides a graphical representation of how the numerical model estimates production for a column of water for a particular instant (or integrated interval of time). Panel 1 of this figure represents the surface irradiance changes over the course of a hypothetical day, as measured by a recording sensor. Panel 2 illustrates the attenuation of light with depth, based on a set of irradiance-depth measurements. The model assumes that the percentage of light at a given depth remains diurnally constant while the absolute values change. Panels, 3, 4 and 5 illustrate the production versus irradiance functions for representative samples from the epi-, meta- and hypolimnion respectively, as determined from incubations at five irradiances each. The model links these measured relationships, via the common parameter, irradiance, to produce the set of production versus depth relationships, shown in panels 6, 7 and 8, for the epi-, meta- and hypolimnion at that particular time.

Thus, from panel 1, for a particular time (t), the surface irradiance (I_0) can be determined and a vertical attenuation profile through the water column constructed (panel 2). Absolute values of irradiance at each depth are calculated relative to I_0. This irradiance versus depth curve spans the epi-, meta- and hypolimnion when surface irradiance is high, as in this example. As surface irradiance drops toward zero ($I_0 \rightarrow 0$), the maximum depth of light penetration moves upward through the meta- and epilimnion toward the surface.

For each depth, a corresponding irradiance value can be determined from the curve in panel 2. This irradiance value can be projected horizontally to determine a corresponding production value for one or more samples in panels 3 through 5. In practice, however, only the epilimnion curve (panel 3) will apply to epilimnion depths, the metalimnion curve (panel 4) to metalimnion depths and the hypolimnion curve (panel 5) to hypolimnion depths.

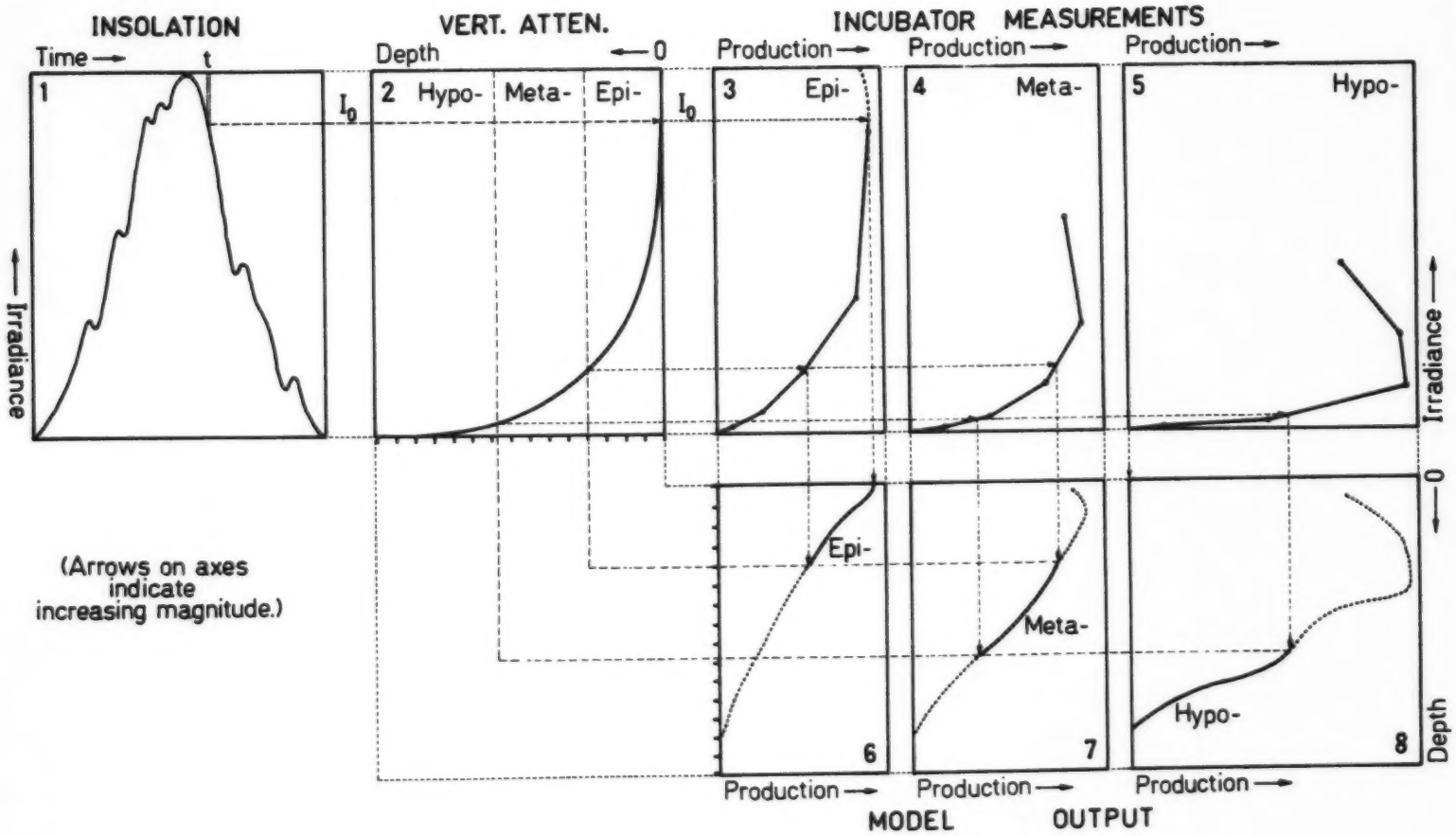


Fig. I.B.1: Graphic representation of the E.L.A. numerical production model for a single time interval. Production vs depth through a three-layer euphotic zone is estimated. (Modified from Fee 1973b).

Thus, for each depth in the water column at any given time, a corresponding value can be determined from the appropriate incubator-produced production versus irradiance curve (panels 4, 5 and 6). The resultant production versus depth relationships are plotted in panels 6, 7 and 8. Only the portion of each curve lying within the applicable depth range (i.e. the solid portion of the line) is used in the integration process to calculate column production. A series of these single time interval, production-depth integrals can be calculated and summed to produce an integrated value for any time interval. If desired, we can correct the column production integrals for basin morphometry by multiplying the production at each depth by the basin area at that depth and summing the resultant products.

The traditional approach in our incubator-models (Fee 1973b, 1977) was to construct photosynthesis-irradiance curves (panels 4, 5 and 6) by interpolating between the actual values measured in the incubator. While mathematically simple, these models required large computers to do the many individual calculations necessary for integrations over longer time intervals.

Fee (1984) has developed a new model which uses calculated inputs of PBm and alpha (Bannister 1974; Jassby and Platt 1976) to produce the photosynthesis-irradiance curves (panels 4, 5 and 6). PBm can be defined as the light-saturated photosynthetic rate per unit chlorophyll. Alpha (α) is the slope (per unit chlorophyll) of the photosynthesis-irradiance curve at subsaturating irradiances. These parameters are determined from the empirical incubator data by means of an iterative curve-fitting process. Chlorophyll concentration data are required for the calculation of the PBm and alpha values. This model utilizes a simplified format for data input and it is designed to run on small personal computers. Fee (1984) has tested the output extensively against that of the previous model. There were no significant differences in the annual integrals.

Regardless of which version of the model one employs, the field sampling and laboratory procedures remain the same, and are detailed in the following sections.

II. CURRENT METHODOLOGY

A. SAMPLING

1. Practical considerations

The size, location, trophic status, and horizontal, vertical and temporal homogeneity of water bodies all determine the number of lakes which can be studied. Our current incubator (Appendix I) permits the simultaneous incubation of up to six lake samples, in duplicate, at each of five light intensities, at any one time and temperature. Laboratory preparation, incubation and processing of these samples require approximately five hours.

In general, our lakes are small (<60 ha) and have homogenous horizontal distributions of phytoplankton but enormous vertical variations (Fig. II.A.1). One central station will adequately characterize the water column. We sample the water column from the surface to the depth at which 0.5% of

surface irradiance occurs. A fluorometer can be used to define the chlorophyll-depth profile (Fee et al. 1977; Fee 1978b) and sampling depths selected accordingly. However, because such equipment is difficult to use routinely, we have adopted simpler sampling guidelines. The depth range to be sampled is subdivided by temperature into layers, each of which is sampled separately. An integrated sample of each layer is taken, as described below.

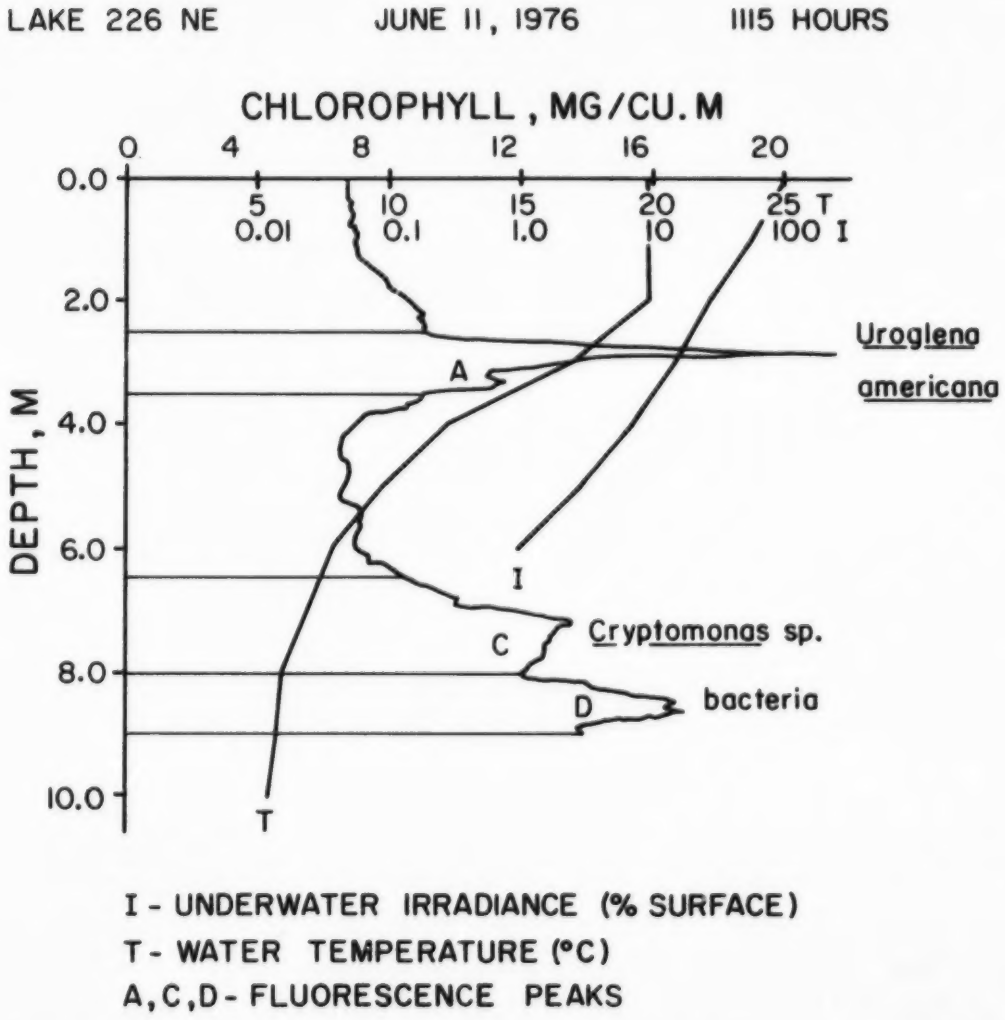


Fig. II.A.1: Profile of an E.L.A. water column showing vertical stratification of algae.

The uppermost sample is taken from the epilimnion, which we define as extending from the surface to a depth at which temperature first shows a change of $1°C \cdot m^{-1}$. The second sample is taken from the metalimnion, wherein temperature changes greater than $1°C \cdot m^{-1}$ occur. In clear lakes, a hypolimnion sample is taken from those depths below the metalimnion where temperature changes are less than $1°C \cdot m^{-1}$ and light levels exceed 0.5% of surface values.

In most oligotrophic lakes, monthly sampling provides adequate estimates of annual production. Eutrophic lakes may have to be sampled weekly to obtain estimates of equal precision, particularly when algal surface blooms are present.

In some eutrophic systems, with low dissolved inorganic carbon (DIC) concentrations, daily production may exceed the concentration of DIC in the lake, being supplemented by invasion of CO_2 from the atmosphere. For such ecosystems, no bottle method can give reliable results and diurnal changes in DIC, oxygen or pH are more appropriate indicators of productivity (Schindler and Fee 1973).

2. Sampling procedure

We use an integrating sampler (Shearer 1978, Fig. II.A.2) for all phytoplankton production sampling. This device permits sampling to be initiated at any desired depth. It fills gradually as it is raised and lowered through a depth stratum. The sampler accepts, with only minor alterations, a variety of glass and plastic sampling containers. We routinely use containers consisting of 2.5 L polycarbonate or glass bottles encased in closed-cell foam insulation with an outer shell of polyvinylchloride (PVC). These containers maintain the samples at *in situ* temperatures during transport, while also protecting light-sensitive algae from surface light levels (Fee 1976). Polycarbonate bottles are lighter and less susceptible to breakage than glass. They also are less likely to cause metal contamination in dilute, offshore marine systems (Fitzwater et al. 1982). However, the walls of these plastic containers tend to collapse under hydrostatic pressure when sampling is initiated at depths greater than about 8 meters. Therefore, rigid-wall sample containers (e.g. glass jugs) are necessary for deep metalimnetic and hypolimnetic sampling with our integrating sampler. Any direct contact of the sample with metal surfaces must also be avoided. In one test, where a sample was passed through 5 cm of copper tubing before incubation, photosynthesis was reduced by 29% compared to controls.

If the stratum being sampled is thoroughly mixed, such as a typical epilimnion on a windy day, an integrating sampler should not be necessary. However, we strongly recommend its use in stratified water columns, particularly in the metalimnion and hypolimnion (Fee 1976), or in epilimnia where pseudovacuolate cyanophytes occur.

Because all samples in a given incubator run must be incubated at the same temperature, we sample from the same thermal zone in multiple lakes on any given day. With only one available incubator, it is usually convenient to sample epilimnia from up to six basins on one day, metalimnia from the same basins on the next day and hypolimnia on the third day. Of course, metalimnion and hypolimnion samples need be taken only from the thermally stratified lakes where the depth of the euphotic zone exceeds the depth of the epilimnion.

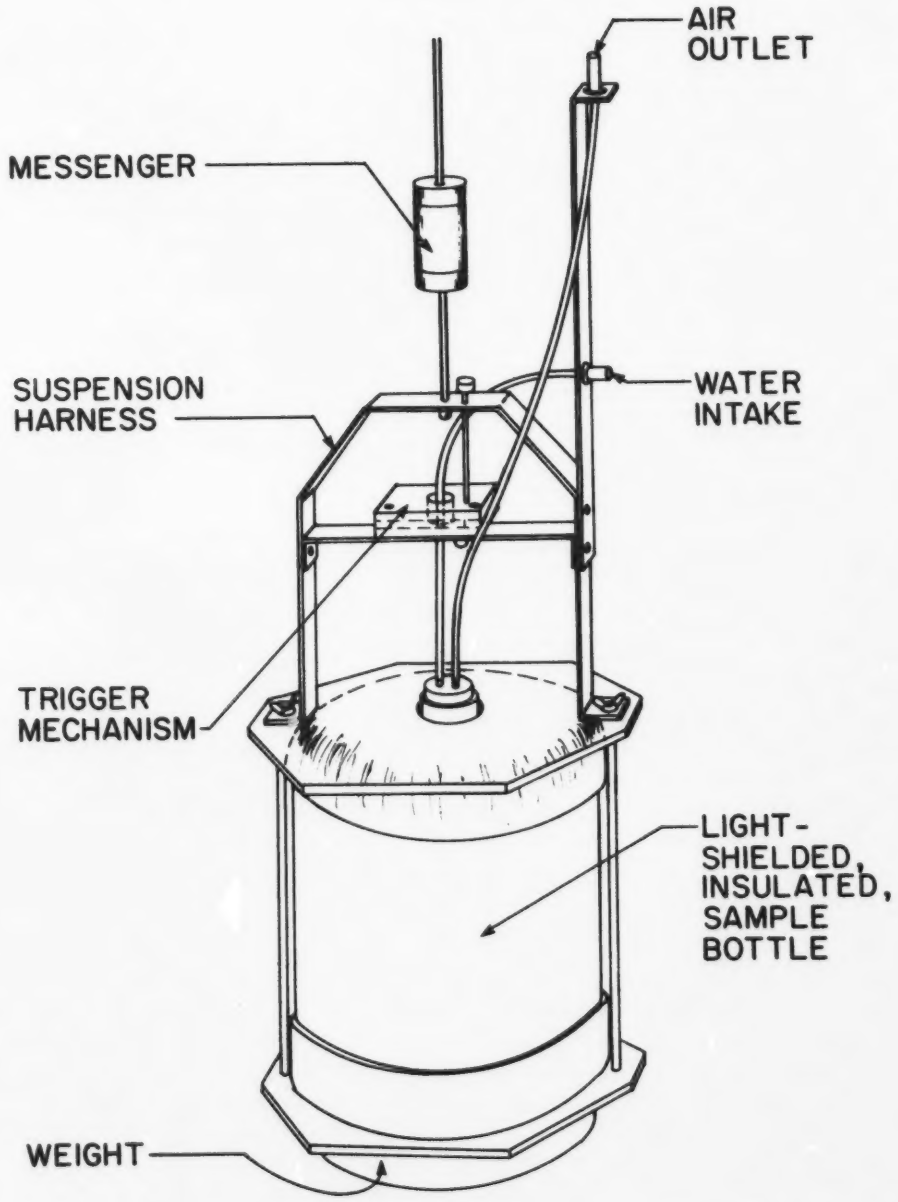


Fig. II.A.2: Integrating sampler.

Once filled and brought to the surface, the insulated, light-tight container is removed from the sampler harness and capped for transport to the laboratory. Although we have found that these samples can be retained for up to eight hours without detriment to the algae, it is advisable to process the sample as soon as possible.

For each sample, the sampling location, date, time, depth range, and any related temperature and light data are recorded. Meteorological data, including cloud cover, wind speed and direction, and precipitation are usually noted, along with any unusual observations or occurrences.

The above procedures apply to sampling of open waters during the ice-free season. At the Experimental Lakes Area, ice and snow cover during most of the winter season excludes measurable light and algal photosynthesis is negligible. However, wherever snow cover is sparse enough to permit light penetration, significant production may occur. A section dealing with procedures for such under-ice sampling is included later in this manual (Section II.F).

B. INCUBATOR MEASUREMENTS

1. Sample preparation

Upon arrival at the field laboratory, lake samples are processed as quickly as possible under low light conditions to avoid light shock. All of the necessary equipment is prepared and organized in order that sample holding time is kept to a minimum.

Prior to use, the incubation bottles are acid washed with concentrated hydrochloric acid, rinsed five times with tap water, three times with distilled water and allowed to dry overnight. Paired incubation bottles are held together with colour coded collars (see Appendix 1, Fig. AP.4) and are acclimated in a water bath to the anticipated incubation temperature. As the samples arrive they are assigned a colour code. The collection times, temperatures and depth intervals are recorded on a laboratory worksheet.

Concern has been expressed in the literature with regard to the type of incubation bottle that is used in primary productivity studies. Fitzwater et al. (1982) found that traces of heavy metals from glass bottle walls caused a reduction in potential productivity rates. Transparent plastic incubation bottles did not show this problem and were therefore considered more suitable for marine productivity studies where ambient metal concentrations are very low. Smith and Baker (1980) and Worrest et al. (1980) found that optical properties of glass bottles could influence potential productivity rates. Quartz glass was found to be the most suitable, followed by Pyrex® and then Wheaton®. The prohibitive cost of large numbers of quartz glass bottles dictates the general use of Pyrex® glass bottles for our work. Significant differences in productivity rates obtained in Pyrex® and polycarbonate bottles have not been found in our studies (E. R. DeBruyn and J. A. Shearer, unpublished data).

The water from the sample bottles is subsampled from the bottom using a siphon tube to reduce turbulence which might alter initial DIC concentration.

Subsamples taken from each 2.5 L lake sample include six pairs of 60 mL incubator samples, a 200-500 mL sample for determination chlorophyll *a* and suspended carbon concentrations, a 100 mL sample preserved with approximately 1 mL Lugol's solution (Kling and Holmgren 1972) for algal identification and biomass determination (Vollenweider 1974) and a 60 mL sample for dissolved inorganic carbon (DIC) analysis. Both the DIC bottles and the incubation bottles are narrow-necked with ground glass stoppers. The syphon tube outlet is inserted to the bottom of each incubation bottle and the bottle is allowed to overflow approximately once its volume after being filled. Chlorophyll *a* concentration is determined using a methanol extraction (Stainton, unpublished) method and suspended carbon content is determined as described by Stainton et al. (1977).

2. Inoculation with carbon-14

The filled incubation bottles are inoculated with approximately 300 µL of radioactive carbon stock solution (~2.0×10^5 becquerels) using a continuous pipettor fitted with a plastic cannula sufficiently long to reach the bottom of each incubation bottle (Fig. II.B.1). The inoculations are done with the incubation bottles in a containment tray to minimize laboratory contamination due to spillage of water containing radioactive material. An alternate method for inoculating the water in the incubation bottles is to add radioactive carbon to a "batch" volume of lake water sufficiently large that the "labelled" water can then be siphoned to each incubation bottle. This "batch" method simplifies the absolute activity determination (see Section 5. Preparation of radioactive carbon standards, p. 12) by minimizing the variability inherent in the dispensed volume of repeating injection equipment and in bottle volumes. However, containment and the potential for laboratory contamination problems are increased with the "batch" method as spillage and overflow of labelled water from the siphon tube occurs when filling incubation bottles. The individually inoculated bottle method therefore presents fewer contamination problems than does the "batch" inoculation method.

A three way comparison was made of productivity rates calculated from incubated sets inoculated with 1) the "batch" method, 2) the individually inoculated method using a glass and stainless steel device, and 3) the individually inoculated method using a glass and plastic device. The results showed no major differences in calculated production rates between methods. They also showed no inhibition of uptake due to toxic trace metals which may be associated with stainless steel equipment.

Once inoculated, the incubation bottles are immediately stoppered. Five of the six bottle pairs are placed on five separate sample wheels of the light incubator (see Section II.B.6 and Appendix 1). The sixth pair, the "dark" bottles, is placed in the end of the incubator farthest from the light source. The entire procedure of subsampling, inoculation and loading the incubator takes less than ten minutes.

3. Dissolved inorganic carbon analyses

A new method for DIC analysis has been adopted to replace the gas-chromatography (Stainton 1973) and conductimetric (Stainton et al. 1977) methods. The newer method (Herczeg and Hesslein 1984) employs an infra-red (I.R.) gas analyzer coupled to a variable span strip chart recorder (Fig. II.B.2). A known volume of lake sample (0.25 - 3.0 mL) is injected into a

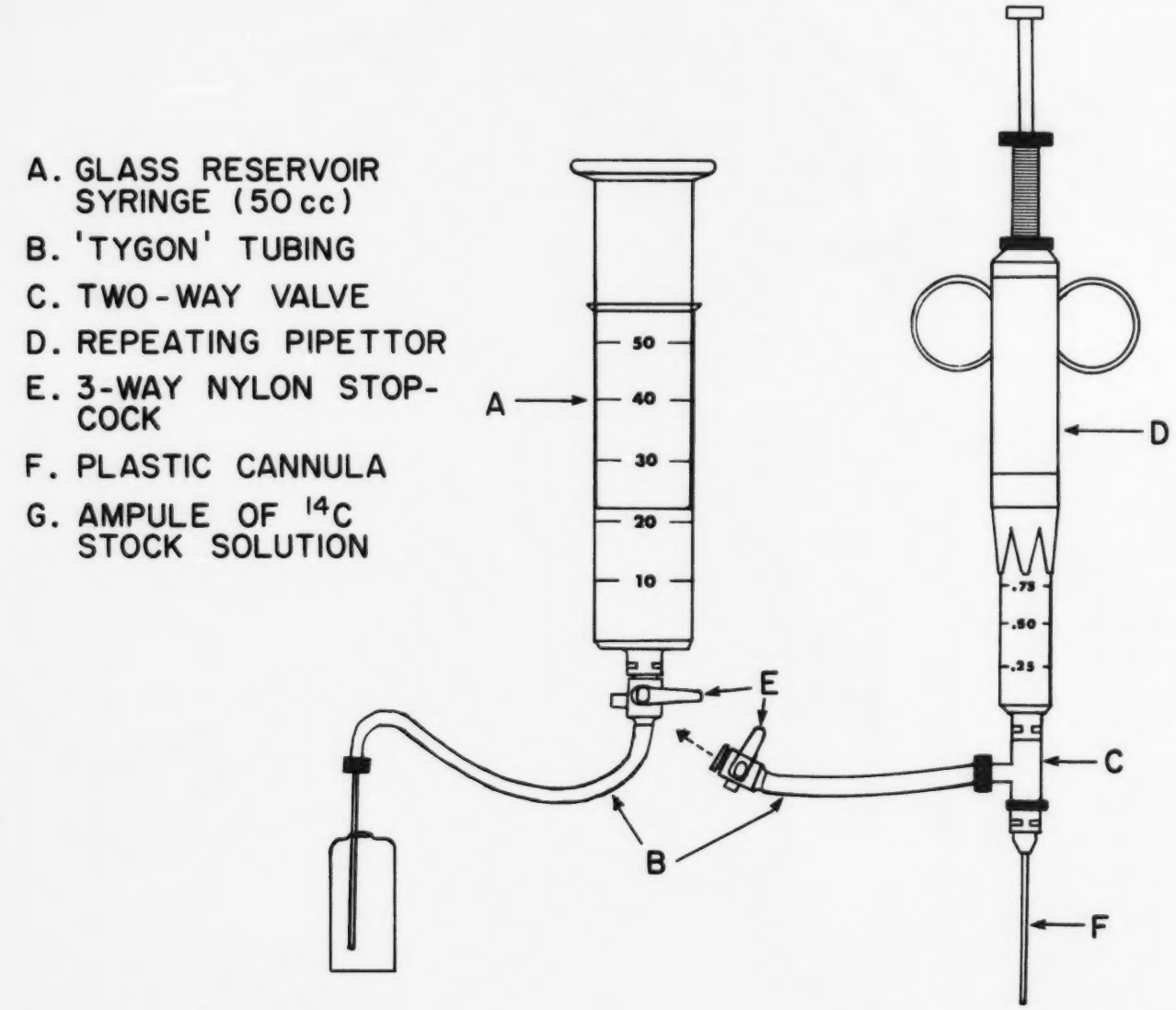


Fig. II.B.1: Apparatus used for filling the ^{14}C reservoir syringe from ampules and for attaching the reservoir to the pipettor.

stripping column along with 0.5 mL of 0.1 N H_2SO_4 or HCL, converting all of the DIC to CO_2. The CO_2 is carried from the stripping column to the I.R. analyser via a CO_2 free carrier gas such as ultra high purity (UHP) nitrogen. A standards curve for analysis may be derived either by injecting liquid standards accompanied by acid or by injecting various volumes of CO_2 gas of known concentration. This method has given excellent results for the range of DIC concentrations (20 μ mole$\cdot L^{-1}$ to 5 m mole$\cdot L^{-1}$) encountered at the Experimental Lakes Area.

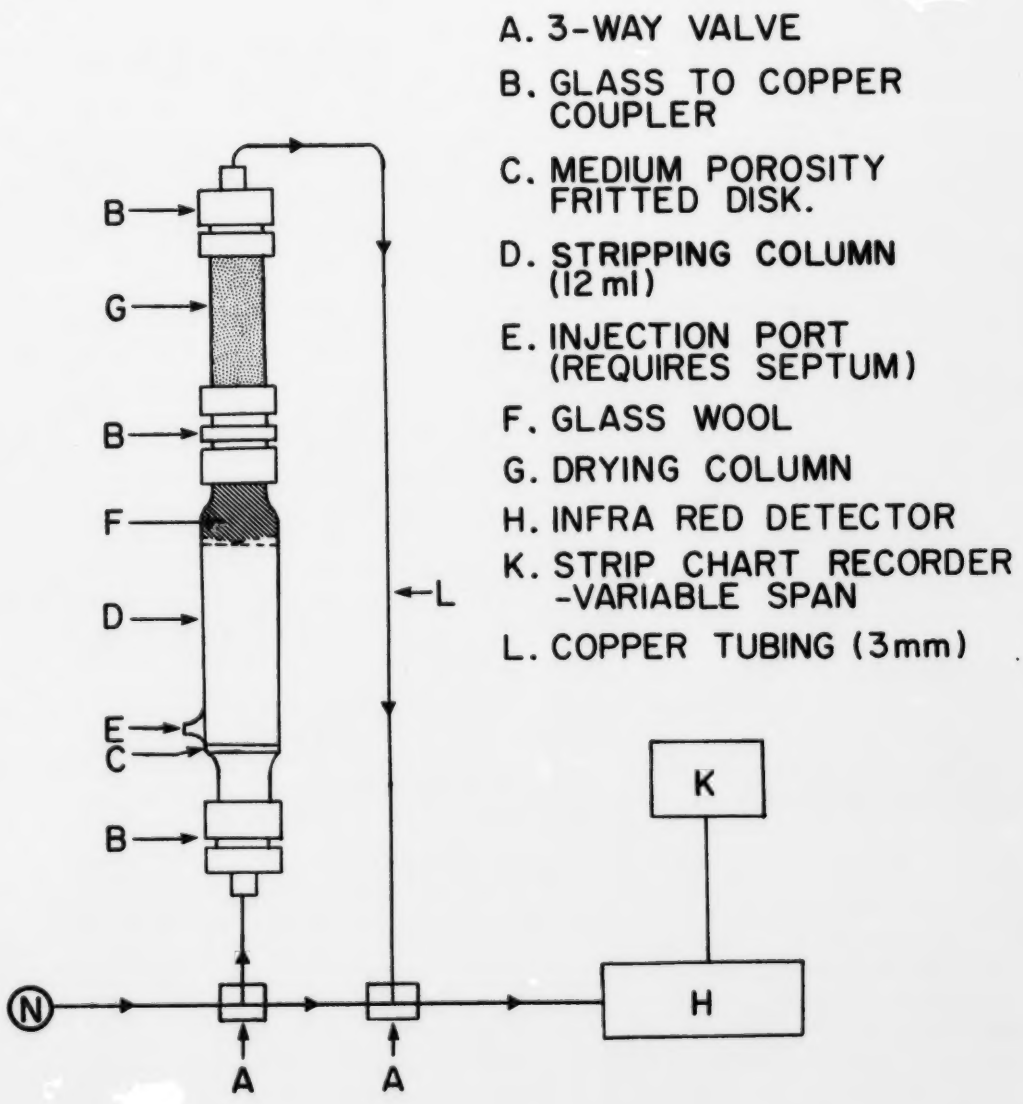


Fig. II.B.2: Schematic diagram showing the stripping column apparatus used in the determination of DIC concentrations.

4. Preparation of stock carbon-14 solutions

Radioactive carbon is obtained as ($Na_2{}^{14}CO_3$) in approximately 3.7×10^9 becquerel lots (100 millicuries). This concentrated "labelled" source is diluted in distilled, deionized water to an activity suitable for daily use ($4.5 - 6.0 \times 10^5$ becquerels per millilitre). The diluted stock solution is stored in flame sealed, autoclaved 20 mL ampoules. A 30 or 50 mL glass syringe with a ground glass plunger and barrel serves as the daily reservoir for the "labelled" solution. The reservoir is attached to the repeating pipettor (Fig. II.B.1). The reservoir may be filled from the ampoules without excessive air entrainment or spillage by attaching a long cannula to the reservoir via Tygon® tubing and a three-way stopcock as shown in Fig. II.B.2. Ideally, if the anticipated daily required volume is contained in the same reservoir syringe, the operator can be assured that all incubation bottles will receive the same amount of activity, facilitating easier calculations of absolute activity through use of a standard series described below.

5. Preparation of radioactive carbon standards

Originally, five 10 µL aliquots of ^{14}C daily stock solution were counted as the standards for absolute activity determination (Shearer and Fee 1974). Beginning in 1979, a more direct method of taking ^{14}C standards was adopted. In order to measure the ^{14}C available for photosynthetic uptake, aliquots equal in volume to those taken from the light bottles are taken from the dark bottles prior to acidification and bubbling. By taking the standards from an inoculated bottle, one is measuring, rather than calculating, the activity added and differences in counting efficiencies are not a problem (Gachter and Mares 1979). Identical volumes of standards and uptake samples also simplify production rate calculations.

Using a suitable pipette, a carbon dioxide trapping agent (100 µL of CO_2 Met® (Amersham)), is placed into each liquid scintillation counting vial. A 5.0 mL aliquot of inoculated water is transferred from the incubation bottle to the vial using a transfer pipette. A suitable volume of a water-compatible scintillation fluor is then added and the vials are immediately capped. Using this approach, the absolute activity of each incubation bottle may be determined if desired. However, this is usually unnecessary because activity from a random sampling of any five incubation bottles typically yields a coefficient of variation of only two percent. The average of five or more standards is routinely used for a day's samples, provided that all of the labelled inoculant for that day came from the same well-mixed reservoir.

The choice of a suitable liquid scintillation fluor depends on the users' requirements. Originally, a dioxane-based fluor was used at ELA (Schindler 1966) but it has since been replaced with a xylene-based fluor (PCS® (Amersham)) for reasons of laboratory safety and convenience. Other large-volume water-compatible fluors giving high counting efficiencies are also commercially available. To maintain high efficiencies, manufacturers recommendations for correct water to fluor ratios and temperature should be followed. Typically, 30% of the total water-fluor solution is water in ELA samples, i.e. 5 mL H_2O in 12 mL fluor when laboratory temperatures range between 20-23°C. Care should be taken to ensure that the relative volumes of water and fluor do not produce a non-countable two-phase condition.

6. Incubation

A description of the incubator currently employed at E.L.A. is found in Appendix 1. The incubator is located in a darkened laboratory in order that all sample preparation may be carried out under low light conditions. The configuration of the incubator allows the operator to process six lake water samples, with five sets of replicate light botties and 1 set of dark bottle replicates each, for a total of 72 bottles.

Incubation temperatures are kept within ±1°C of the actual lake sample collection temperatures either with a thermostatically controlled refrigeration unit (see Appendix 1) or by adding ice or warm water to the incubator. The duration of the incubation normally ranges from 2-4 hours, depending on the available DIC and productivity of the lake. At the end of the incubation period, the bottles are removed from the sample wheels and transferred to the processing laboratory in a light-tight box.

Photosynthetically active radiation (PAR) at each sample wheel of the incubator is monitored daily with a Biospherical Instruments® QSP-200 quantum scalar irradiance sensor and meter. Similar measurements have also been obtained with the Biospherical Instruments® QSL-100 sensor and meter and the LI-COR® LI-193S spherical quantum sensor attached to a LI-185 meter (DeBruyn and Shearer 1981). Spherical sensors are necessary for making incubator light measurements because a substantial portion of the light at each sample wheel is reflected. Tests have shown that cosine response sensors may underestimate light in the incubation chamber by as much as 80% (DeClercq and Shearer 1979).

With the aid of a holding jig, the spherical light sensor is supported by bottle-holder posts (Fig. AP.4a) on each wheel. The spherical sensor is thereby positioned in the plane of the path traced by the rotating bottles and facing the light source. A light reading is taken at each of the sample wheels and recorded.

The light gradient of the incubator may be modified through the use of filters, as described in Appendix 1, to approximate the natural light climate from which the integrated lake sample is taken . As the light attenuation varies with depth of sample and time of year, there will be a need to closely monitor and adjust the light intensity for each incubation.

7. Post incubation processing

Incubator samples are prepared for ^{14}C uptake assay using the principle of acidification and bubbling described by Schindler et al. (1972). Originally, the process required the use of six sets of ten 30 millilitre Allihn tubes. We now use a variation of an apparatus described by Wessels and Birnbaum (1979). This vacuum chamber apparatus (Fig. II.B.3) consists of a 15.25 centimeter (inside diameter) acrylic tube secured between an acrylic base and top plate by a threaded rod and wing nut. An internal supporting plate is designed to hold twelve 22 millilitre scintillation vials for the six replicate incubator samples. The top plate has twelve small drilled holes aligned with those of the supporting plate. Twenty gauge needles, 3.8 centimeters long, are held by Eppendorf® plastic pipette tips which seat securely in the holes of the top plate. Each needle tip is suspended so that it reaches to

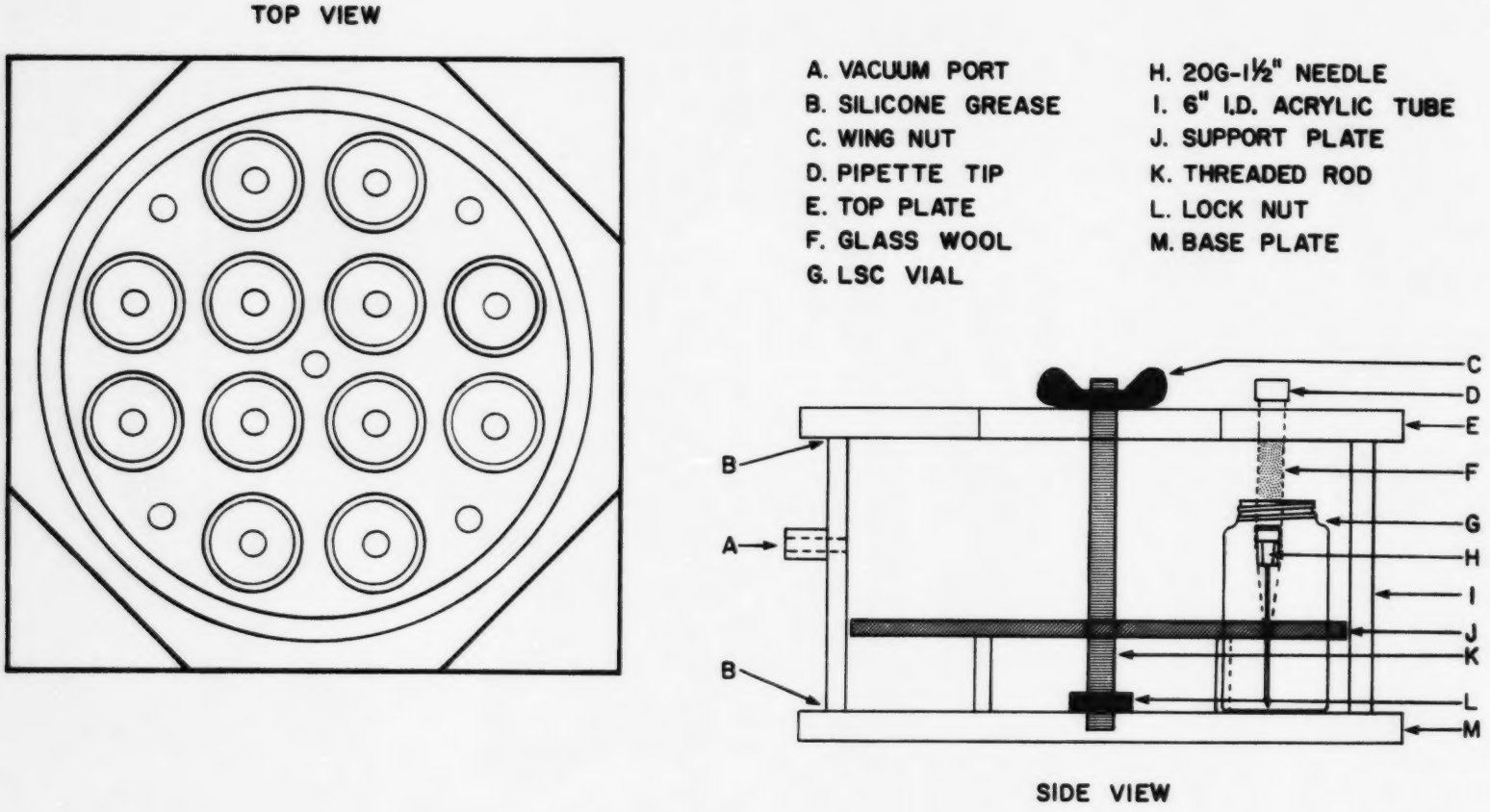


Fig. II.B.3: Vacuum bubbling chamber for removal of unassimilated 14-C carbon from productivity samples.

the bottom of the scintillation vial when the top plate is secured to the chamber. Five millilitre aliquots of water from the incubation bottles are placed directly into polyethylene or glass scintillation vials containing 0.5 mL of 0.1 N HCl. [Note: The concentration of acid required is dependent on the alkalinity of the sample. The pH during bubbling should be in the range from 3.0-3.5.] These vials are then enclosed in the vacuum chamber which is evacuated using an air pump.

Approximately 12 kPa of vacuum is applied to the side port of the sealed chamber causing air to be drawn down through the 12 needle/pipette bubblers and bubbled through the liquid in the scintillation vial. The air passes from the chamber through a filtration flask trap to the suction port of the pump. Air from the pump is directed either to a fumehood or scrubbed through a strong base solution such as sodium hydroxide, to trap radioactive carbon. A valved manifold in the suction line allows the operation of more than one chamber at any given time.

Care in the choice of needle gauge and amount of vacuum applied to each chamber is necessary. Larger gauge needles (e.g. 16) cause the liquid to bubble too vigorously while smaller gauge needles (e.g. 25) present problems in bubbling consistency due to particles getting trapped in the tip of the needle. Too high vacuum may cause bubbling irregularities, resulting in a loss of activity due to splash.

The advantages of a vacuum chamber over an Allihn tube method as described by Schindler et al. (1972) are the elimination of the need to process samples in a fume hood, reduction in processing steps, reduction in error potential caused by handling frequency, reduction in cost of apparatus and a simplified operation for the clean-up of radioactive substances on laboratory apparatus.

Tests on E.L.A. water have shown that, after bubbling for 5 minutes with the pH adjusted to approximately 3.0-3.5, less than 1% of the unassimilated radioactive carbon solution remains (Fig. II.B.4). To ensure the maximum removal of the unassimilated labelled solution the samples are bubbled routinely for 15-20 minutes. Efficiency of inorganic carbon-14 removal has not been tested in a wide variety of lake types and should be tested for each new application.

After bubbling, the top plate holding the bubbling needles is removed and the scintillation fluor is added. The vials are capped, marked and stored in a darkened area for at least one hour. This period of dark adaptation reduces possible counting errors brought about by chemiluminescence, photoluminescence or static electricity (Kolb and Horrocks 1981).

8. Liquid scintillation counting

The limitations and advantages of various types of scintillation vials have been discussed by Painter (1973). Linear polyethylene vials are usually used at ELA, when counting is done within 24 hours of processing, as they allow more efficient counting of carbon-14 than glass vials. The more costly glass vials are used when counting cannot be done within 24 hours, as they are impermeable to xylene and toluene. By comparison polyethylene vials show weight losses ranging from less than 1% to over 7% per day (Painter 1973).

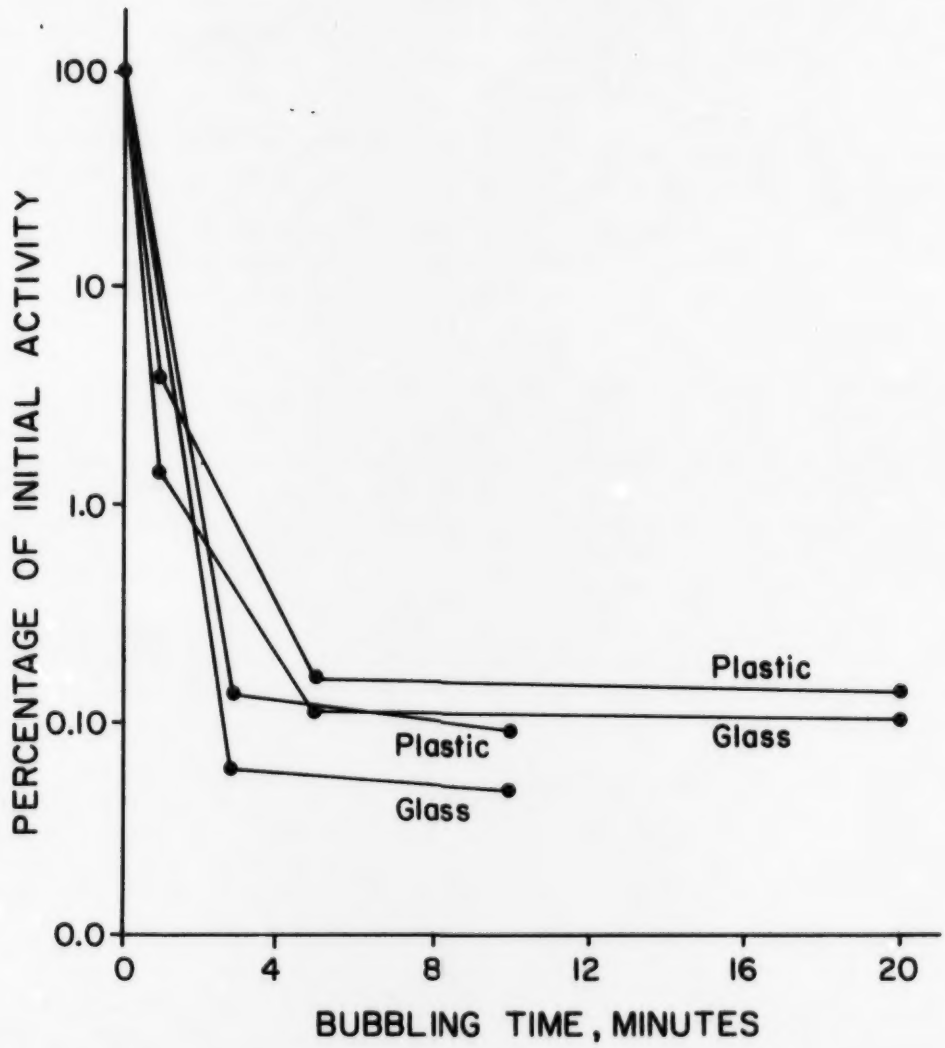


Fig. II.B.4: Bubbling time versus percentage activity remaining. Each point is the mean value for 5 replicates. In all cases, plastic vials show a slightly greater percentage remaining than do glass vials.

Sondergaard (1980) discussed some problems with carbon-14 adsorption to polyethylene vials but this has not been found to be a problem in this methodology. A comparison of the carbon-14 remaining in plastic vials versus glass vials following normal lake sample incubations and processing indicated there was no significant difference attributable to vial type in a paired-sample test ($n = 24$, $P = 0.05$). Others have found major differences in productivity rates determined from experiments when comparing glass and polyethylene vials. It is therefore recommended that tests be done to determine which vial type is most suitable for the experiment being conducted.

When possible, all samples, including ^{14}C standards and quenched standards should be counted within 24 hours of sampling, using a liquid scintillation counter. We use a Beckman® LS-8000 or LS-2800. For these counters, counting efficiencies are determined by the "H number (H#)" method (Horrocks 1977), employing a ^{137}Cs external standard plus a sealed set of quenched standards. Any good external standard quench correction method will also suffice. The quenched standards in use at ELA use the same fluor as in the samples, plus varying amounts of carbon tetrachloride as a quenching agent, to obtain a range of "H" numbers for a quench curve. Counting efficiencies range from 80% to 92% for most samples.

The statistical accuracy to which the samples are counted should be be chosen depending on the degree of accuracy required by the investigator. Two minute counts are felt to be sufficient for the higher activity ^{14}C and quenched standards while the unknown lower activity samples are counted to 10 000 counts or 70 minutes, whichever comes first.

9. Calculation of production rates

The rate of inorganic carbon uptake by the algae during the incubation is based on the assumption that:

[B1]
$$\frac{^{12}C \text{ uptake}}{^{12}C \text{ available}} = \frac{^{14}C \text{ uptake}}{^{14}C \text{ available}}$$

The ^{12}C uptake may be calculated knowing: pre-incubation DIC concentration (^{12}C available), activity of ^{14}C standards (^{14}C available) and the activity of the bubbled aliquots (^{14}C uptake). By incorporating a correction for dark bottle uptake of ^{14}C, isotopic discrimination and incubation time, one may use the following equation to calculate production rates.

[B2]
$$P_{net} = \frac{(R_L - R_D) * C * 1.06}{T * A}$$

where P_{net} is assumed to be the net rate of inorganic carbon uptake in mg $C \cdot m^{-3} \cdot h^{-1}$.

R_L is the activity remaining in acidified and bubbled light bottle aliquots in DPM.

R_D is the activity remaining in acidified and bubbled dark bottle aliquots in DPM

C	is the total carbon available for uptake in mg $C \cdot m^{-3}$ C = [DIC] $\mu M \cdot L^{-1}$ * 12 (atomic weight of carbon)
T	is the sample incubation time in hours
A	is the activity of ^{14}C added to the incubation bottles in DPM (from ^{14}C standards).
1.06	is an isotopic discrimination factor for ^{14}C, as it has a greater atomic weight than ^{12}C.

A desk-top computer is used to calculate the photosynthetic rates. A program calculates the quench curve and uses that curve to convert the counts per minute (CPM) from the scintillation counter to disintegrations per minute (DPM). Many modern liquid scintillation counters will do this calculation internally and output the results as DPM. Once the DPM have been obtained, the photosynthetic rates may be calculated using equation B2.

C. IN SITU LIGHT ATTENUATION

1. Practical considerations

As with the incubation samples, the optimum frequency for measuring vertical light attenuation (extinction) is dependent on the trophic state of the lake. Oligotrophic lakes change little in transparency over the season and monthly profiles of light attenuation are adequate. Weekly profiles may be necessary in eutrophic lakes where the rapid wax and wane of algal blooms cause light attenuation to be more variable. Variable turbidity caused by river inflows or resuspension of sediments in shallow lakes could also create a requirement for more frequent in situ light measurements.

The time of day and the cloud conditions during the period of measurement are also critical to reliable estimates of light attentuation (Fig. II.C.1). Both solar angle and cloud cover affect the rate of light attenuation, particularly in highly transparent lakes (Currie 1961; Booth 1976; Combs 1977; Hojerslev 1978). Combs (1977) found that underwater light attenuation measurements made in mid-morning (under a solar altitude of 30°-35°) produced a minimal net error when used with Fee's (1977) model to estimate photosynthesis. We therefore schedule our water column light measurements for mid-morning (or mid-afternoon) whenever possible. We also prefer to take our readings under either a clear sky or a uniform cloud cover. An overcast sky acts as a solar diffuser and the solar angle is less critical when readings are taken under overcast conditions. Windless conditions are preferred because wave action makes light measurements taken just below the surface very erratic.

Many types of light measuring devices are available. For photosynthesis studies, light should be measured in units of total quanta within the photosynthetically active portion of the spectrum, 350 nm or 400 nm to 700 nm (Federer and Tanner 1966; Jerlov and Nygard 1969; Tyler 1973; Chapman and Campbell 1975; Hojerslev 1978). Light attenuation in the lakes, incubator irradiances and surface solar radiation should all be measured using compatible sensors for photosynthetically active radiation.

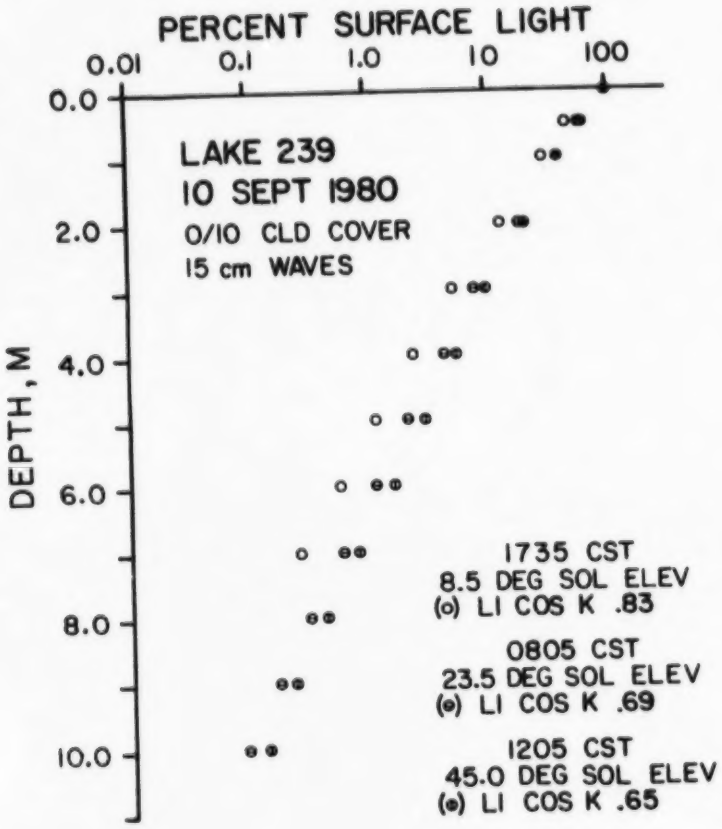


Fig. II.C.1: Variation in vertical light attenuation with changing solar elevation.

Booth (1976), Combs (1977) and Hojerslev (1978) recommend the use of a spherical (4π) collector for all underwater light measurements pertaining to algal photosynthesis. A spherically collecting sensor responds to irradiance incident on a point from all directions (scalar irradiance) and should best approximate the response to light of an algal cell. We agree that a spherical quantum sensor should be employed for measuring incubator irradiances and for any other underwater measurements where absolute irradiance levels are required. However, our methodology requires only depth-irradiance values expressed relative to the surface irradiance measured in air.

We have compared 4π spherical sensors with flat-plate cosine-corrected (2π) sensors for determining in situ profiles of relative irradiance versus depth (Shearer and DeClercq 1980). No significant differences are apparent in the relative responses of the two sensors for underwater profiling in the E.L.A. However, if a spherical collector is used for light profiling, a problem arises in relating the underwater irradiances to the "in air" surface irradiance. Because of its design, a spherically collecting sensor, when used in air above the surface, will respond to direct sunlight (regardless of solar angle), to skylight and to light reflected from the water surface (Fig. II.C.2). Particularly at low solar elevations, this can produce a serious overestimate of the irradiance actually entering the surface of the water column. Surface wave action and spherical sensor design often make it difficult, if not impossible, to precisely measure irradiances just below the surface.

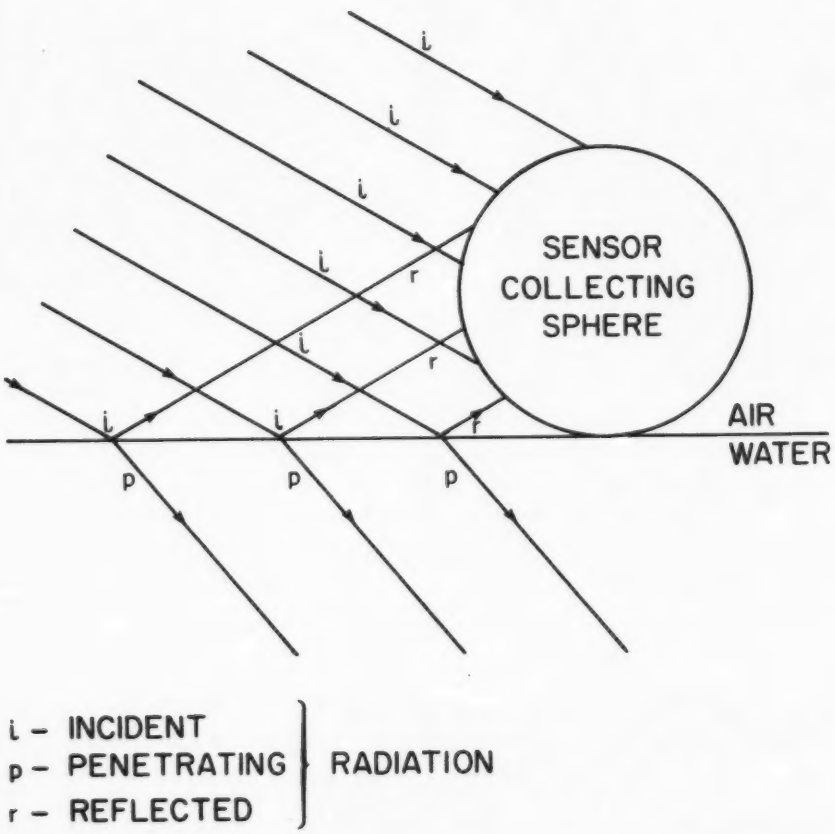


Fig. II.C.2: Spherical (4π) collector above water surface in direct sunlight.

The cosine sensor offers a more practical solution to lake surface irradiance measurement. The reduced response of the cosine sensor to direct sunlight at low solar elevations tends to compensate for the smaller fraction of incident light entering the water column because of increased surface reflectance. "In air" cosine surface readings agree well (±5%) with cosine readings made just below the surface under calm conditions (provided that the sensor immersion factor is applied).

Therefore, if a spherical collector is used to determine vertical light attenuation, the surface value should either be measured with a cosine sensor calibrated to the same standard or be back-calculated from readings taken below the surface.

When surface light conditions are variable because of patchy cloud cover, a surface reference sensor (deck cell) should be used. Underwater readings can then be adjusted to a standard value of surface light.

The problems associated with solar angle, surface reflectance, cloud cover, sensor design and immersion effect make the accurate determination of in situ irradiances a complex task. Relating surface irradiance to irradiances beneath the surface can be particularly difficult. Booth (1976) has described a hemispherical sensor design for measuring quantum surface solar radiation (Biospherical Instruments Inc.®, model QSR, San Diego, CA.). While this instrument addresses some of the problems inherent in other collector designs, some correction must still be made for average daylight reflected at the water surface (Hojerslev 1978).

Although accurate measurement of irradiance levels is desirable, even large differences in light levels produce relatively small differences in estimates of integral plankton production using Fee's model. In one computer simulation, we numerically increased light levels within the water column of a lake (L226NE, 1978) by 21%, but the calculated production (integrated over the entire ice-free season) increased by only 6%. In a second simulation experiment, we numerically increased incubator light levels by a mean of 68% but kept the carbon uptake values from the incubator constant. The calculated differences in annual integral production, as estimated by the model, were 14% in a lake with low transparency (L227, 1979) and 18% in a high transparency lake (L223, 1979).

This "buffering" effect apparent in the integral production estimates suggests that a large fraction of E.L.A. phytoplankton photosynthesis occurs under non-limiting light conditions (i.e. during mid-summer, mid-day and in epilimnia). Production potential is usually lower during the fall and hypolimnetic production peaks occur within relatively small portions of the total lake water volumes. Thus, light controlled production represents a relatively small portion of the integrated total and the photosynthetic potential of the algae is the key factor driving the model.

2. Measurement procedure

We currently employ a cosine-corrected, flatplate underwater quantum sensor for in situ light attenuation measurements (LI-COR® LI-192S). A similar quantum sensor is used for surface reference and both sensors are connected to a meter via a "deck to sea" switch module. Both sensors measure

photons within the 400-700 nm waveband (Biggs et al. 1971) and have fully cosine-corrected responses.

The underwater sensor is mounted in a triangular, transparent acrylic plate which is horizontally suspended at the end of a metered cable (Fig. II.C.3). This arrangement permits the sensor to point vertically upward while being suspended at a known depth.

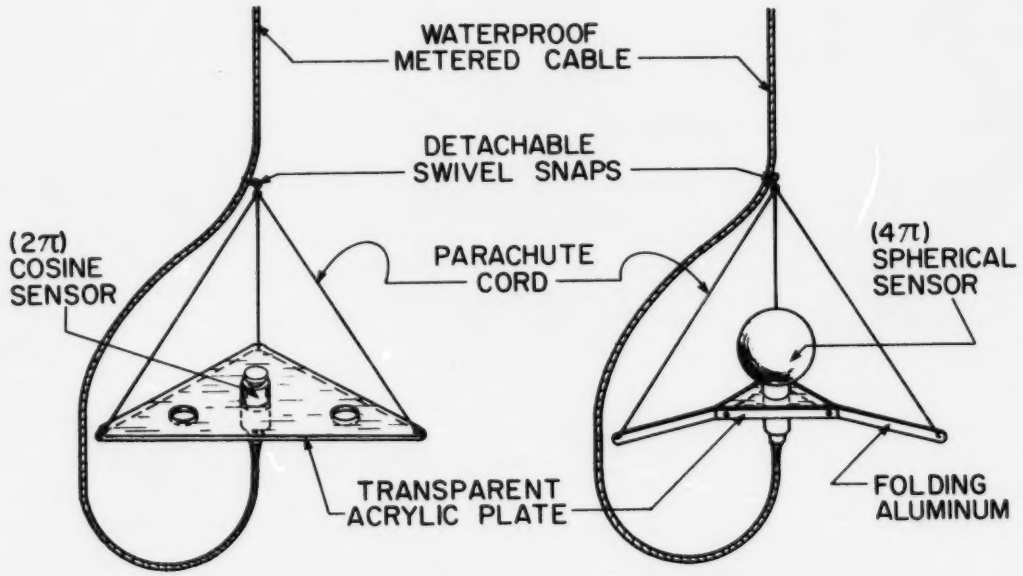


Fig. II.C.3: Two simple suspensions for <u>in situ</u> profiling with quantum sensors.

We have detected minimal backscattering of light in the E.L.A lakes. Thus upwelling irradiances are insignificant and we can use a cosine sensor pointed upward. However, backscattering may be significant in turbid lakes. Either a 4π collecting sensor could be employed or additional cosine readings could be taken with the sensor pointing downward.

If required because of variable cloud cover, the LI-190S surface reference sensor is mounted in a level, unshaded position in the boat. The following description of procedure assumes that the reference sensor is being used.

An initial reading with the underwater sensor is taken in air above the lake surface on the unshaded side of the boat. Care must be used to ensure that light reflecting from the side of the boat does not affect the reading or that a shadow from the suspensor harness does not fall upon the sensing surface. A comparative reading is taken immediately from the reference sensor (deck cell) under the same sky conditions. This is the initial surface reference reading to which all later ones will be compared.

The underwater sensor is then lowered beneath the surface to a depth of 0.25 or 0.5 meters and the new reading recorded. Again, care must be used to avoid either shading the sensor or exposing it to reflected light. A comparative reference sensor reading is also recorded.

Readings are taken at intervals through the water column until the available light is 0.5% or less of the surface irradiance. We routinely make readings at 1.0 meter intervals, although smaller depth intervals may be necessary in shallow, turbid or eutrophic basins. Surface readings are taken each time an underwater measurement is made. Other parameters, including location, date, time and meteorological conditions also should be recorded.

If sky conditions are uniform and the surface irradiance does not fluctuate significantly during the profiling period, the surface reference cell is not required. An initial surface reading with the profiling sensor will suffice. If in doubt, it is wise to recheck the surface irradiance at the conclusion of the profile.

As already noted, it is not necessary to use absolute units for calculating the vertical attenuation. These absolute units change with solar elevation and cloud cover. It is necessary only that all measurements be made relative to the surface irradiance measured in air. This surface irradiance will later be equated to the calibrated values of surface solar radiation described in the next section (Section D).

Underwater readings made with a given sensor are lower than air readings made with the same sensor because of the "immersion effect" (Jerlov and Nygard 1969; Smith 1969; Westlake 1965). The exact value of the immersion factor depends on sensor design and is normally determined by the manufacturer. It is either 1.34 or 1.4 for our LI-192S sensors. Thus, to make the surface reading compatible with the underwater values, it is divided by the correct immersion factor:

[C1]
$$U_0 = \frac{U_A * R_0}{F * R_0} = \frac{U_A}{F}$$

where U_0 is the underwater sensor reading at the surface, corrected for immersion effect.

U_A is the underwater sensor reading in air above the surface.

F is the immersion factor for the underwater sensor.

R_0 is the initial surface reference reading.

Some instruments incorporate a switch which automatically calibrates the instrument for taking readings in air or in water. If such a device is present, care must be exercised to ensure that it is always correctly positioned when taking readings.

To correct the in situ irradiance-depth values for fluctuations of the surface insolation while the profile is being measured, each underwater sensor reading is multiplied by the ratio of the initial reference reading to the reference reading for that depth.

$$[C2] \qquad \frac{U_z * R_0}{R_z}$$

where U_z is the underwater sensor reading at depth Z.

R_0 is the initial surface reference reading.

R_z is the surface reference reading taken at the time U_z was taken.

Therefore, the percentage of surface irradiance reaching any depth Z (% $SURF_z$) is calculated as follows:

$$[C3] \qquad \% \ SURF_z = \frac{U_A * R_0 * 100}{U_0 * R_z}$$

If the surface irradiance did not change significantly during the profile, equation C3 becomes

$$[C4] \qquad \% \ SURF_z = \frac{U_z * 100}{U_0}$$

When using a spherical collector for profiling, we calculate a value for U_0, for reasons discussed in Section II.C.1. Such a value for U_0 can be back-calculated from the underwater data, making some empirically determined allowance for the increased dispersion and attenuation of light in the upper few centimeters of the water column.

D. SOLAR RADIATION MONITORING

At the Experimental Lakes Area, we continuously monitor the surface insolation by means of a cosine-corrected, flat plate quantum sensor (LI-COR® LI-190S) with direct input to a strip chart recorder. The sensor is identical to that used as a surface reference "deck cell" for irradiance versus depth profiling. It is calibrated in millivolts per 1000 microeinsteins (1 microeinstein = 6.023×10^{17} quanta). To eliminate shading from nearby trees, the sensor is located atop a tower at the E.L.A. field camp. Our most remote study lake is approximately 16 km from the tower.

The data are recorded continuously during the ice-free season. These surface irradiance records include periodic time references which can be entered either manually or automatically. The insolation data are digitized and processed for input to the model (Appendix 2).

The output from our solar sensor can also be fed directly into an analog/digital converter-integrating module which will print digital integrated solar values for predetermined time intervals. Various companies now offer integrating units which magnetically or electronically store integrated data.

The surface solar radiation data are a critical input for the calculation of precise integral production values. It is therefore advisable to have some back-up system for collecting this data.

Various other sensors can be used in emergency situations, provided the calibration is known. We have experimented with a hemispherical solar reference sensor (Biospherical Instruments® QSR-240). However, this instrument also measures that portion of incident light which would normally reflect from the lake surface. We recommend caution in the use of insolation data from such a sensor.

The surface solar irradiance values and the incubator irradiance values (Section II.B) must be absolute. All the quantum sensors are supplied with factory calibrations referenced to a National Bureau of Standards (N.B.S.) standard. Manufacturers usually recommend annual recalibration, though we have found the LI-COR® sensors to change very little. We use a precision power supply and spectral standard lamp to periodically check the responses of our sensors. If a change is noted in a sensor's output, it is sent to the factory for recalibration. We also compare the empirical integrated outputs for cloudless days to the theoretical cloudless totals for those days. This procedure allows us to check our calibrations from one year to the next and helps to ensure consistent results.

E. DATA PROCESSING

1. Introduction

With the continuing development of smaller computers and improved data processing equipment, methodology in this area is in a state of flux. This section describes, in general terms, the basic procedures for using the numerical model to calculate integral production and related parameters. The details will vary with the available data handling facilities and with the numerical model being used.

We process the data as soon as possible following the completion of field data collection and incubator experiments. Rapid data handling enables us to evaluate experimental results and make any required procedural modifications quickly. If necessary, critical samples can be retaken or experiments repeated before conditions change significantly. In studies of dynamic natural systems, such speedy evaluation is essential to ensure that significant gaps in the data set are avoided.

2. Preliminary calculations and storage

The equations employed for reducing raw transparency and incubator data have been described already. These calculations are routinely performed, using desk-top computers, within 24 hours of the measurement or sampling time. Reduced data are verified and stored on magnetic disks for future use in the numerical model.

Printed data summaries are produced for both the vertical attenuation profiles (Table II.E.1) and the incubator samples (Table II.E.2). Plots of irradiance versus depth (Fig. II.E.1) and incubator production versus

irradiance (Fig. II.E.2) are also produced for each data set. These summaries provide a ready reference from which to evaluate the success of each incubation and to determine whether more frequent incubations are required or whether the incubator light regime requires adjustment. The vertical attenuation profile is also used to ascertain the depth of the euphotic zone and thus to determine the maximum sampling depth for incubation samples.

Table II.E.1: Printout of data set used to construct Fig. II.E.1 (above). Depth is in meters. Time is when sample was taken. Temperature is in ^{o}C. Units of dissolved inorganic carbon are $\mu moles \cdot L^{-1}$. Units of suspended carbon and chlorophyll are $\mu g \cdot L^{-1}$. I1-I4 are irradiances. P1-P4, in replicate, are uptake values.

DATE	DEPTH	TIME	TEMP.	DIC	SUSP-C	CHLOR	I4	P4	I3	P3	I2	P2	I1	P1	C.V.(%)	NOTE
16 MAY	0.0-10.0	0815	6.0	99	940	6.9	13	.87 .75	48	3.24 3.18	167	5.49 5.69	876	5.24 5.51	4.28	5,6

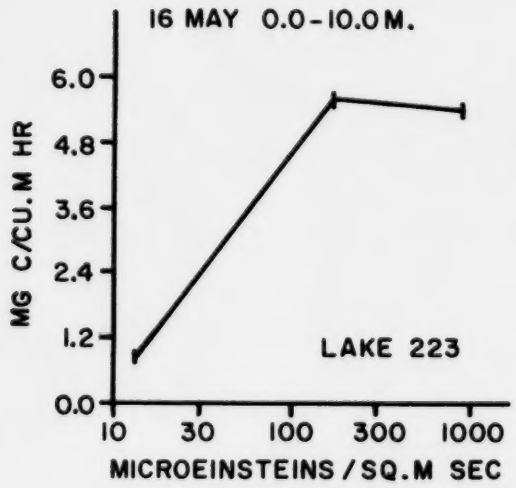


Fig. II.E.1: Sample plot of carbon uptake (mg $C \cdot m^{-3} \cdot hr^{-1}$) versus incubator irradiances ($\mu Ein \cdot m^{-2} \cdot sec^{-1}$) for one lake sample incubated at four intensities. Vertical bars indicate the range of replicate measurements.

Table II.E.2: Printout of data for depth-light profile plotted in Fig. II.E.2 (above).

LAKE 223

DATE: 16 MAY TIME: 0830 HOURS
ATTENUATION COEFFICIENT: .54 R**2: .9958

DEPTH	%SURF.	DEPTH	%SURF.	DEPTH	%SURF.	DEPTH	%SURF.	DEPTH	%SURF.
0.00	100.00	0.50	69.52	1.00	48.28	2.00	27.03	3.00	14.00
4.00	8.01	5.00	4.63	6.00	2.80	7.00	1.55	8.00	0.99
9.00	0.62	10.00	0.38	11.00	0.25	12.00	0.16		

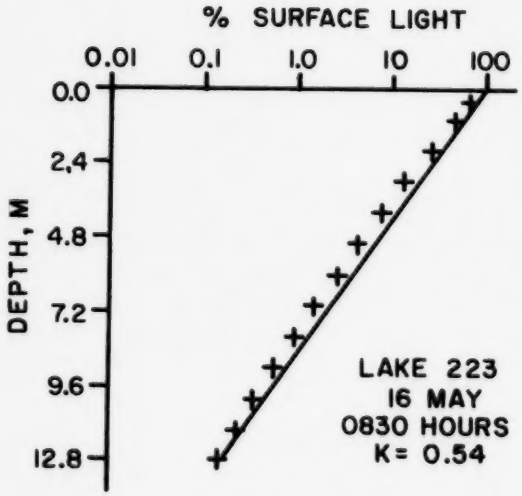


Fig. II.E.2: Sample plot of percent surface irradiance versus depth for one in situ profile The slope (-K) of the depth-light curve is calculated, then forced through an intercept at 100%, 0m. (Vollenweider 1974).

3. Calculation of integral production

This final step in our procedure for estimating integral phytoplankton production requires the use of a numerical model, as described in Section I.B. Developed over a period of more than 15 years, this model, in its various versions (Fee 1973a, 1977, 1984), has been applied to more than 150 lake-years of data from E.L.A. The model has also been applied to data from Southern Indian Lake (a large, impounded, sub-arctic lake) (Hecky and Guildford 1984), Lake Tanganyika (Hecky and Fee 1981), arctic lakes, prairie pothole lakes, and an arctic polynia. Fee (1979, 1980) and Fee et al. (1982) have presented some of the integral data from the E.L.A. produced with this technique.

The model, when implemented on a suitable digital computer, calculates integral production over any time period for which data are input. Earlier versions (Fee 1977) were written in FORTRAN and were implemented on large, "mainframe" computers. The latest version (Fee 1984) is written in PL/1® and can be run on any microcomputer utilizing the CP/M® or MS-DOS® operating system. Complete instructions for using this model are given by Fee (1984).

A simplified, BASIC language version of the model is provided in Appendix 2. This program permits the calculation of daily integral primary production using any small computer programmable in BASIC. Necessary input data are: incubator irradiances and carbon uptake measurements, irradiance-depth measurements, and surface irradiances over time for the period to be calculated. The program calculates integral production at each of a selected number of depth strata and outputs this value for each stratum, along with the percentage of surface irradiance occurring therein. The integrals from the various strata are also summed to provide a daily integral total, on a column basis, for the waterbody.

F. APPLICATION IN ICE-COVERED LAKES

At ELA, in recent years, we have paid little attention to phytoplankton production under ice. The small lakes studied are buried under at least 25 cm of snow for almost the entire period of ice cover. We have found that winter production is usually less than 5% of the annual total in such lakes, and that most of this occurs in the last month before ice-out, when snow has melted and incident solar radiation is high.

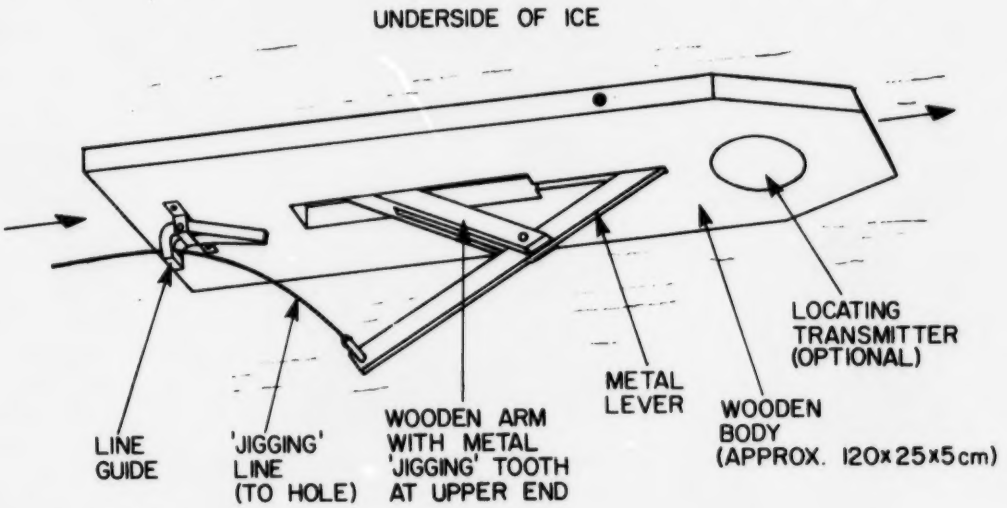
At higher latitudes, or on larger lakes, under-ice production can be a higher percentage of the annual total, because such lakes are often thinly covered or even devoid of snow due to lower snowfall and greater wind exposure. Ice cover is present during long daylight periods (May-June) at high latitudes.

Winter measurements pose special problems. Samples taken under very cold conditions will require protection from freezing. This can be done very simply with a styrofoam-insulated box or cooler containing one or two hot water bottles or polyethylene bottles of warm water.

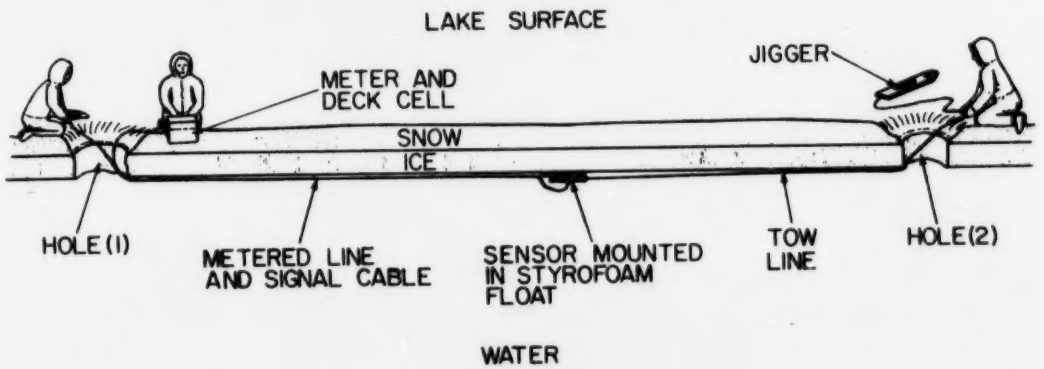
Winter algae are especially sensitive to bright light, and special precautions should be taken to avoid light shock. When possible, sampling should

be done from a light-tight hut, or under a blanket or tarpaulin, or before sunrise. The integrating sampler described in Section II.A minimizes light exposure problems.

Light measurements made under ice require special attention. Near-surface light meter readings taken through a hole in the ice will be affected by light entering the hole. This can be minimized by filling the



a. Prairie ice jigger (used for towing line horizontally under ice from one hole to another).



b. Measurement of light under ice. The float-mounted sensor is positioned at known distances between the holes by means of the metered lines. Simultaneous under-ice and "deck" readings are taken. Snow cover can be measured for each position after the readings are completed.

Fig. II.F.1: Under-ice techniques.

hole with snow. Readings then can be taken in the usual manner. A plot of light vs depth will usually reveal abnormally high near-surface light values, even if the hole is carefully covered. These should be ignored when calculating the extinction coefficient.

The light-field just under the ice is also important and may vary greatly if snow cover is patchy. It is therefore necessary to obtain accurate information on such variation (Reid et al. 1975).

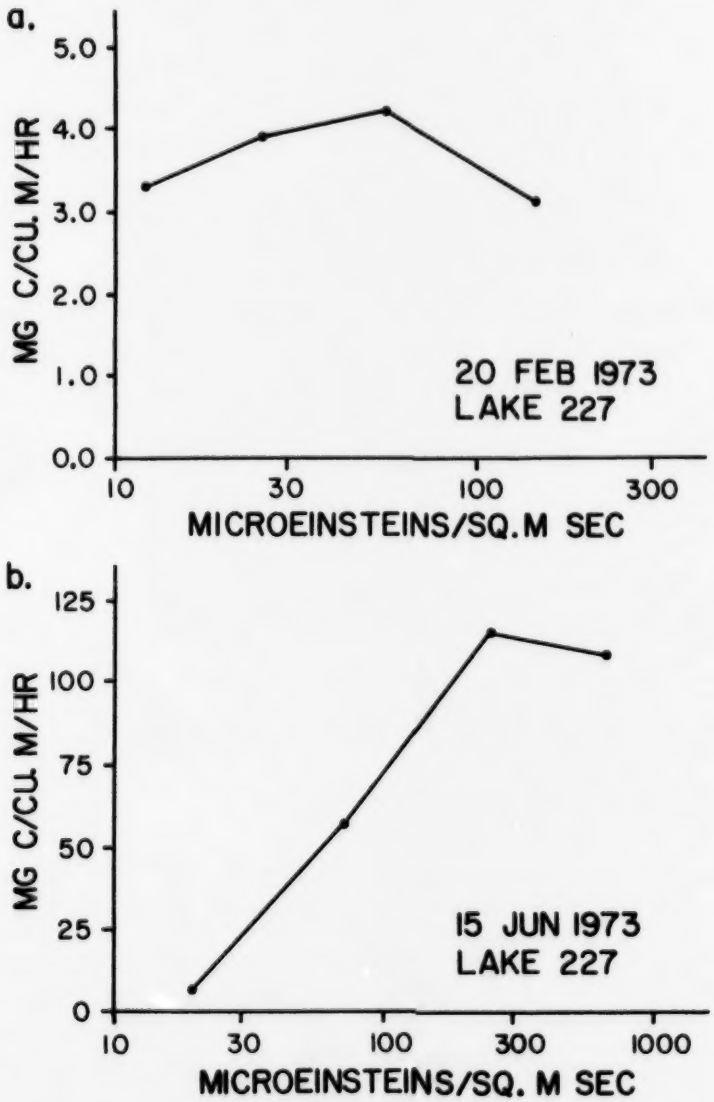


Fig. II.F.2: Plots of carbon uptake versus incubator irradiance for a winter sample (a) and a summer sample (b), showing differences in saturation and inhibition intensities.

In order to perform such measurements, a long line is towed under the ice, using a prairie ice jigger (Fig. II.F.1a), an idea borrowed from northern commercial fishermen (Sprules 1949). The light sensor is mounted in a block of 2" thick styrofoam, which floats against the ice, attached to the line, and is towed along under the ice. Measurements are taken at numerous points, and compared to a deck cell set up on the ice (Fig. II.F.1b). It is convenient to mark the tow line so that corresponding measurements of snow depth on the ice surface can be made. Several transects and many snow depth measurements are necessary to characterize the light field when snow conditions are patchy.

In the laboratory, incubations are made at light intensities and temperatures far lower than those used in summer. Saturation and light inhibition occur at far lower intensities than in summer because of algal adaptation (Fig. II.F.2). Temperatures under the ice will usually range between 0.5 and 4°C, and an efficient cooling system will be required to maintain such temperatures in the incubator.

III. METHODS JUSTIFICATION

A. LIQUID SCINTILLATION COUNTING

1. Physical quenching

Steeman-Nielsen (1977) expressed the fear that physical quenching (quenching due to the inability of the soft beta emissions of ^{14}C to penetrate cell walls of algae or filters) will alter the results of the liquid scintillation method. In fact, tests for this problem are described in the technical manuals of all major manufacturers of scintillation counters, and are necessary for any counting of solid samples by liquid scintillation. We give results here (Table III.A) in response to Steeman-Nielsen's critique.

Twenty 25 mL aliquots were drawn from a large, well-stirred sample of natural lake water (Lake 239) which had been incubated with ^{14}C for four hours. Ten aliquots were filtered onto Millipore® HA membranes, 47 mm in diameter with a pore size of 0.45 µm. The other ten were filtered on to Whatman® GF/C glass fiber filters, 45 mm in diameter.

Five of the Millipore® and five of the GF/C® filters prepared in this manner were placed directly in dioxane-based fluor and counted immediately. The other five Millipore® filters were combusted by ignition in 4-L flasks which had been flushed and filled with oxygen. A scintillation vial in the bottom of each flask contained 5 mL of phenethylamine to absorb CO_2 from combustion. The flask was left sealed for 23 hours following combustion. Calibrations showed 90 ± 0.7 percent of ^{14}C was trapped in this manner. After digestion, 10 mL of toluene fluor was added to each sample for counting.

The remaining five GF/C® filters were cut into pieces and placed in 2 mL of NCS® (Amersham) in scintillation vials. Vials were held at 40-45°C overnight, then a 15 mL aliquot of toluene fluor was added to each vial prior to counting. Preliminary trials showed that when Millipore® filters were dissolved in NCS®, extremely low counting efficiencies resulted, so that the

Table III.A. A comparison of dpm yielded by counting procedures, as described in the text. Sample from Lake 239, June 2, 1970, 1 m depth.

Dioxane & Millipore		Dioxane & GF/C		Toluene + GF/C		Toluene + Phenelthylamine, combusted Millipore	
e.s.c.r.[a]	internal[b]	e.s.c.r.	internal	e.s.c.r.	internal	e.s.c.r.	internal
8125	8170	7950	7906	7936	7996	8240	8200
8172	8160	8046	8040	8102	8098	7860	7820
7945	8032	8036	8260	8036	8072	7748	7876
8101	8046	8128	8144	8112	8096	8080	7940
7936	8050	8190	8200	8028	8008	8076	8040

[a] e.s.c.r. = external standard channels ratio
[b] internal = internal standardization

NCS®-Millipore® combination was not included in our tests. Samples were all standardized by external standard channels ratio. After counting, each sample was spiked with 50 µL (91 191 dpm) of ^{14}C-labelled hexadecane, and recounted, so that external standard channels ratio efficiencies could be compared to internal standardization. All samples were counted to 16 384 counts in a Picker Liquimat 220® counter.

Algae in the samples were composed of Dinobryon sociale var. americanum, D. divergens, Synura uvella, Peridinium aciculiferum, Mallomonas acaroides, M. caudata, M. globosa, Tabellaria fenestrata, Gymnodinium mirabile, Rhizosolenia sp., and Chrysosphaerella longispina.

Counting efficiencies for toluene-phenethylamine and dioxane were 82-85%. Slightly lower values (68-72%) were observed for toluene-NCS®.

No significant differences were observed in dpm yielded by any of the counting or standardization procedures (probability of difference <0.01). One may therefore conclude that the least complicated handling procedure (dioxane fluor without combustion or digestion plus external standardization) was adequate. In particular, physical quenching did not appear to be a significant problem.

In a second experiment, conducted in 1972, we tested 10 mL dioxane fluor plus 5 mL of water plus ^{14}C-hexadecane versus 15 mL dioxane plus ^{14}C-hexadecane. While the water lowered the average counting efficiency from 83 to 69 percent, efficiencies determined by the external standard channels ratio technique yielded values which were indistinguishable from samples without water (probability of difference <0.02).

More recently, we tested water compatible xylene (PCS®, ACS®) and pseudocumene (Beckman MP®) based liquid scintillation fluors using a Beckman® LS2800 counter. The mean counting efficiency of 10 mL fluor plus 50 µL ^{14}C hexadecane was 96.2% ± 0.88. After the addition of 5.0 mL lake water, the average efficiency fell to 91.4% ± 0.60. There was no significant difference found among the tested fluors (probability of difference <0.005).

B. CORRECTION FOR FILTRATION ERROR

There has been considerable controversy in the literature over the correction for filtration error proposed by Arthur and Rigler (1967).

McMahon (1973) found that corrections as high as 20x were artifacts due to retention of DI ^{14}C by filters. It is, however, unlikely that this artifact is the same problem discussed by Arthur and Rigler who rinsed their filters, which should eliminate DI ^{14}C. Their correction factors average less than 2x.

In our tests, the 2x correction factor could not be eliminated by rinsing filters with dilute HCl, or by fuming, so it is certainly not due to DI ^{14}C. Using Sephadex® analysis of filtrates, Schindler et al. (1972) found that, when 1-2 mL of incubated water were passed through a 47 mm diameter, 0.45µ Millipore® membrane, nearly all of the ^{14}C in excess of molecular weight 5000 was retained by the filter. When 50 mL of water was filtered, only a small percentage of the high molecular weight material was retained. Samples

retained. Samples of intermediate volume had intermediate percentages of ^{14}C retained by the filters. Filters simply seemed to become saturated with high molecular weight ^{14}C as increased volumes were filtered.

Because the high molecular weight ^{14}C appears to be of biological origin, it should be included in estimates of total production. In order to obtain such estimates using filtration, the filtration correction procedure must be employed (Fig. III.B.1). However, unless the radioactivity of samples is very high, counting statistics are poor if only small volumes are filtered.

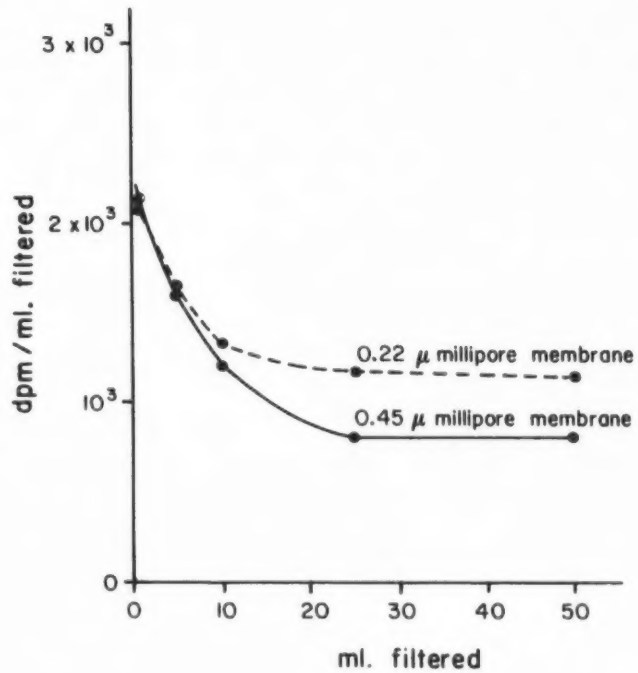


Fig. III.B.1: An example of the filtration error correction curve for phytoplankton production results (from Schindler and Holmgren 1971).

As an alternative to filtration with its inherent correction factor, we have chosen to count the aqueous samples directly with a water compatible fluor after acidifying and bubbling to remove the unincorporated $DI^{14}C$.

Filtration of large (>25 mL) samples appears to give adequate estimates of ^{14}C incorporated into particulate matter alone.

C. EFFICIENCY OF ACIDIFICATION AND BUBBLING

Our results for freshwater, both very soft (E.L.A., 20-150 µM DIC, pH 5.8-7.7) and very hard (Char Lake, 3500-6000 µM DIC, pH 7.6-8.0), and for seawater (Bermuda, Resolute Bay) indicate that bubbling for 10 minutes or more at pH 3.5 removes almost all of the DI^{14}C. Remaining CO_2 is less than one percent of the lowest production values at optimum light which are likely to occur in either freshwater or marine systems. We have, however, found that an occasional batch of isotope purchased commercially may contain particulate or dissolved organic matter contaminated with ^{14}C. If bubbled blanks are unacceptably high, such problems should be checked. Particulate contaminants can be eliminated by filtering the solution. Dissolved organic compounds can be conveniently oxidized in a sealed quartz vessel suspended near a strong ultraviolet light (Stainton et al. 1977).

Some workers (e.g. Hecky and Fee 1981) have encountered difficulties with removal of DI^{14}C in certain waters. The methodology should therefore be carefully evaluated in each study area before embarking on a routine productivity measurement program.

D. INCUBATOR VALIDITY

Advantages of using an artificial light incubator for algal primary production studies have already been noted in Sections I and II (see also Fee 1973a). However, the validity of this methodology is often questioned. Can carbon uptake measured in bottles under controlled, artificial light conditions validly be used to estimate algal productivity in natural waterbodies?

In the early stages of this methodology development, the incubator carbon uptake values were compared with values from "traditional" *in situ* methodology. Agreement between the results of the two methods was good. Indeed, some of the control parameters used in early versions of the incubator-model were selected to improve its agreement with *in situ* results (Fee 1973b, 1975, 1978a).

In 1978, we had a unique opportunity to test the validity of both incubator and *in situ* bottle techniques. An experiment was carried out involving a whole-lake addition of ^{14}C as a tracer for monitoring the carbon budget of the lake (Bower 1981). It was therefore possible, by dawn and dusk sampling of the mixed layer, to directly estimate the epilimnion "whole-lake" primary production on a daily integral basis over a period of weeks.

During a portion of this whole-lake experiment, we were able to conduct incubator-model and *in situ* bottle experiments to estimate epilimnion productivity using these techniques. Incubator-model experiments were conducted according to the methodology then in use (DeClercq and Shearer 1979). The incubator light source at that time was quartz-halogen. The *in situ* incubations were conducted both for 4 hour mid-day periods and 16 hour dawn-to-dusk periods using the method described by Schindler and Holmgren (1971). Water for both incubator and *in situ* incubations was collected with the integrating sampler (Shearer 1978) and all post-incubation processing involved acidification and bubbling and liquid scintillation counting.

The scale and complexity of these comparative experiments have made a detailed interpretation of the results difficult. A paper attempting such interpretation is under review. In general, the incubator-model results were in better agreement with the measured whole-lake integrals than were the *in situ* results. *In situ* bottle integrals were somewhat lower than incubator-model integrals. Often, this lower *in situ* bottle value can be attributed to light inhibition in bottles suspended near the surface on sunny days.

From the results of these experiments, we can conclude that the incubator-model technique is a valid method of estimating epilimnetic phytoplankton production. Both the incubator and the *in situ* methods can provide reasonable estimates of the whole-lake values, but the incubator-model offers more advantages for prediction, and for comparisons of lake to lake and of year to year.

More work remains to be done on the responses of the photosynthesis-irradiance relationship to changes in light quality and to fluctuating light. The effects of changing solar elevation, cloud cover, surface waves and wavelength attenuation coefficients make any attempt to precisely model the natural system overwhelming at best. However, our simplified incubator-model gives good approximations and permits us to compare integral primary production from one lake to another or from one year to another with confidence.

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R. Hecky, R. Hesslein, I. Davies and P. Healey made helpful suggestions during the preparation of the manuscript.

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APPENDIX 1

ARTIFICIAL LIGHT INCUBATOR DESIGN

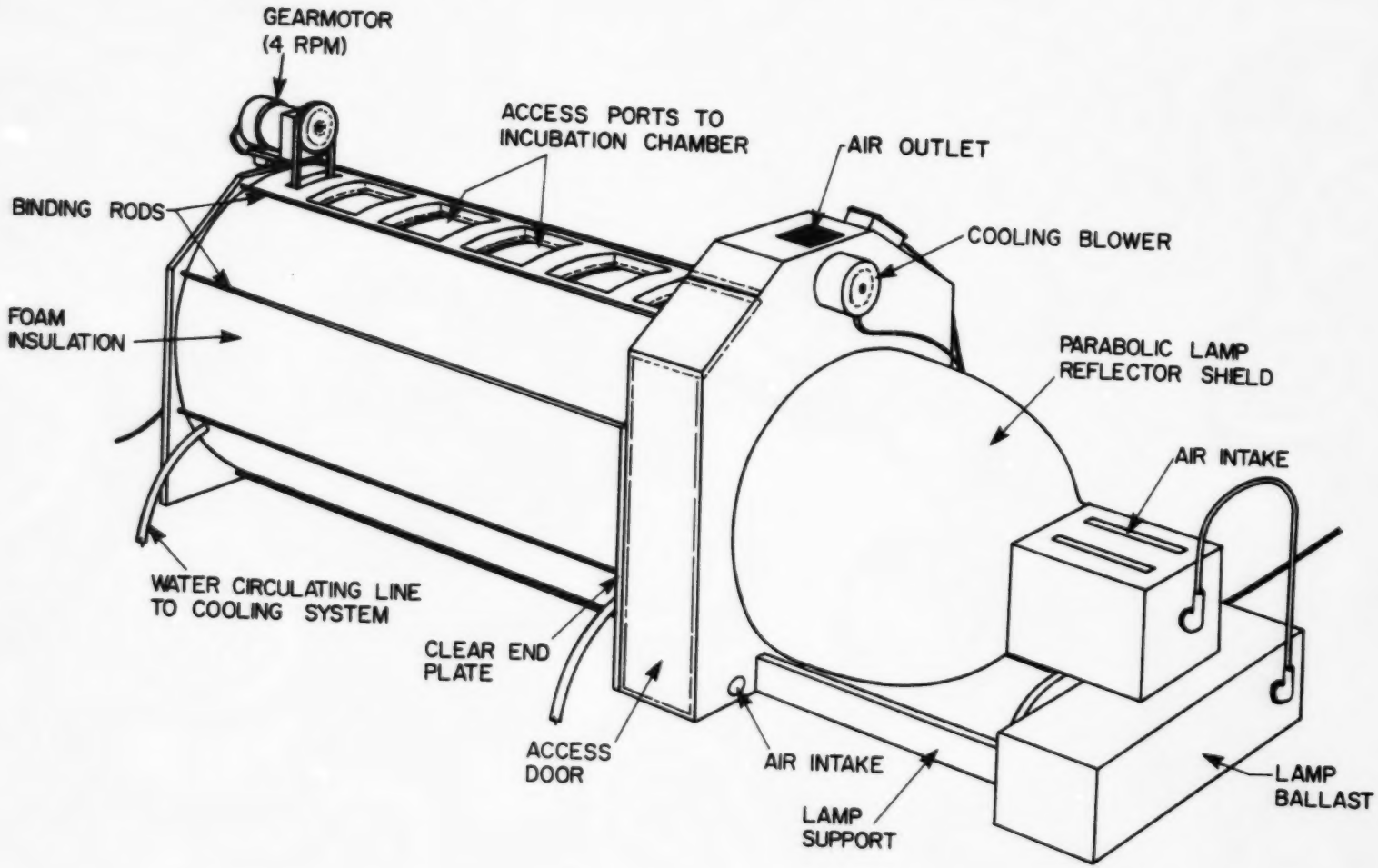
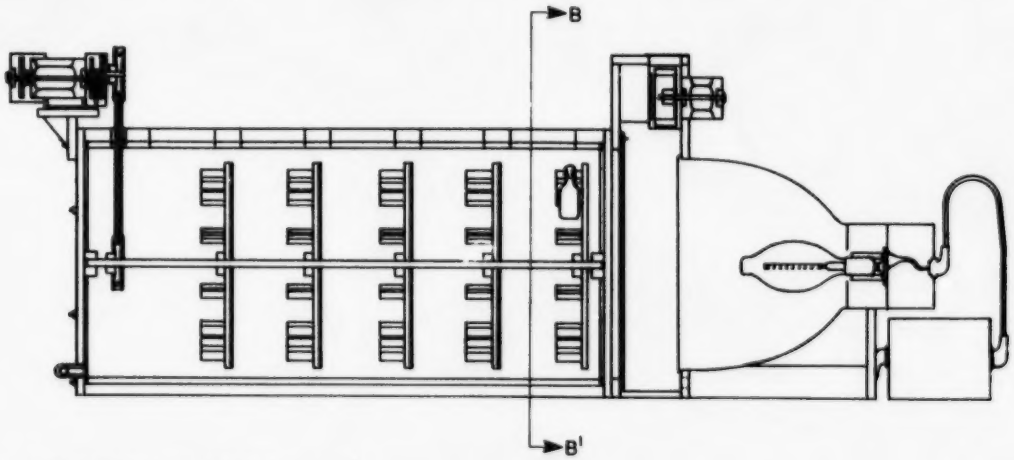


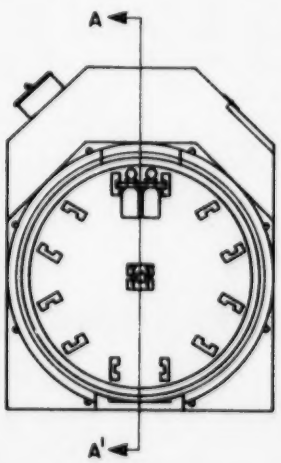
Fig. AP.1: Artificial light incubator.

Fee (1973a) described an incubator used in early studies involving his model. Shearer (1976) gave plans and operating instructions for a more compact "portable" incubator. We have recently constructed and tested an updated incubator design which incorporates most of the best features of earlier designs and provides several major advances. This incubator (Fig. AP.1 and AP.2) is described here.

A single 400 w metal halide lamp (Sylvania® MS400/C/HOR) and parabolic reflector (Sylvania® MAS-118-400) replaces the multiple 500 w quartz-halogen lamps used in earlier designs. This lamp permits incubator irradiance levels equivalent to full sunlight, if required. The spectrum (Figure AP.3) is



a. LONGITUDINAL SECTION A-A'



b. CROSS SECTION B-B'

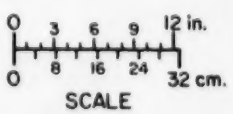


Fig. AP.2: Sectional views of artificial light incubator.

concentrated within the photosynthetically active region with its peak in the 550 to 580 nm range. Despite its high irradiance output, this lamp uses less energy and produces much less heat than do the quartz-halogen lamps. The problems inherent in dissipating excessive heat build-up are largely eliminated.

Metal halide, and other high intensity discharge (HID), lamps require a ballast to produce their correct operating voltage. Thus, it is not possible to vary their output by varying the input voltage. Any variation of incubator irradiance levels must be achieved using neutral density filters of some type. Perhaps the simplest and most effective neutral density filters are layers of ordinary window screening (McAllister and Strickland 1961).

The incubation chamber is a 1 m length of large diameter (45 cm O.D., 1 cm WALL) grey, P.V.C. waterpipe with one end of 1.3 cm thick grey P.V.C. sheet and the other end of 1.3 cm thick clear acrylic sheet. The pipe is clamped between the end sheets using eight brass rods. Gaskets of 8 mm O.D. latex tubing, compressed between the pipe ends and the end plates, provide watertight seals.

Access holes have been cut in the top of the P.V.C. pipe and the pipe has been wrapped in 3 cm of polyethylene closed cell insulating foam (Dow Ethafoam® 100).

Running lengthwise within the pipe is a 1.5 cm diameter stainless steel shaft on which are mounted five rotatable clear acrylic sample wheels. Each wheel is designed to accept six pairs of 60 mL Pyrex® glass reagent bottles (Corning® 1500) as illustrated in Fig. AP.2. A 4 r.p.m. gearmotor (Dayton® 3M321) mounted atop the P.V.C. end plate drives the rotating sample wheel assembly via a single V-belt.

The lamp-reflector is horizontally mounted in a cooling chamber which is itself fastened to the clear acrylic endplate. The positioning is such that the lamp output is directed through the endplate and down the length of the incubation chamber. Filters and diffusers within the cooling chamber and on the fronts of the sample wheels are used to tailor the incubator irradiance regime. The combination of the single lamp and the tubular grey incubation chamber, provides a more uniform light field than did previous incubator designs.

A small blower (Dayton® 4C012) mounted in the top of the cooling chamber, draws air in past the lamp and exhausts heated air at the top. This cooling fan is probably not essential with the metal halide lamp but it does reduce heat build-up. Access to the cooling chamber for filter and lamp changes is provided by a sliding panel on one side.

A drain for the incubation chamber is located at the base of the P.V.C. end plate. If flow-through cooling of the water in this chamber is required, two tube connectors are fitted in the lower wall of the large PVC tube, one near either end. Water is then pumped continuously from one end through a thermostatically controlled cooling coil and back into the other end of the incubation chamber. Uniform mixing within the chamber is provided by the rotating sample wheel assembly.

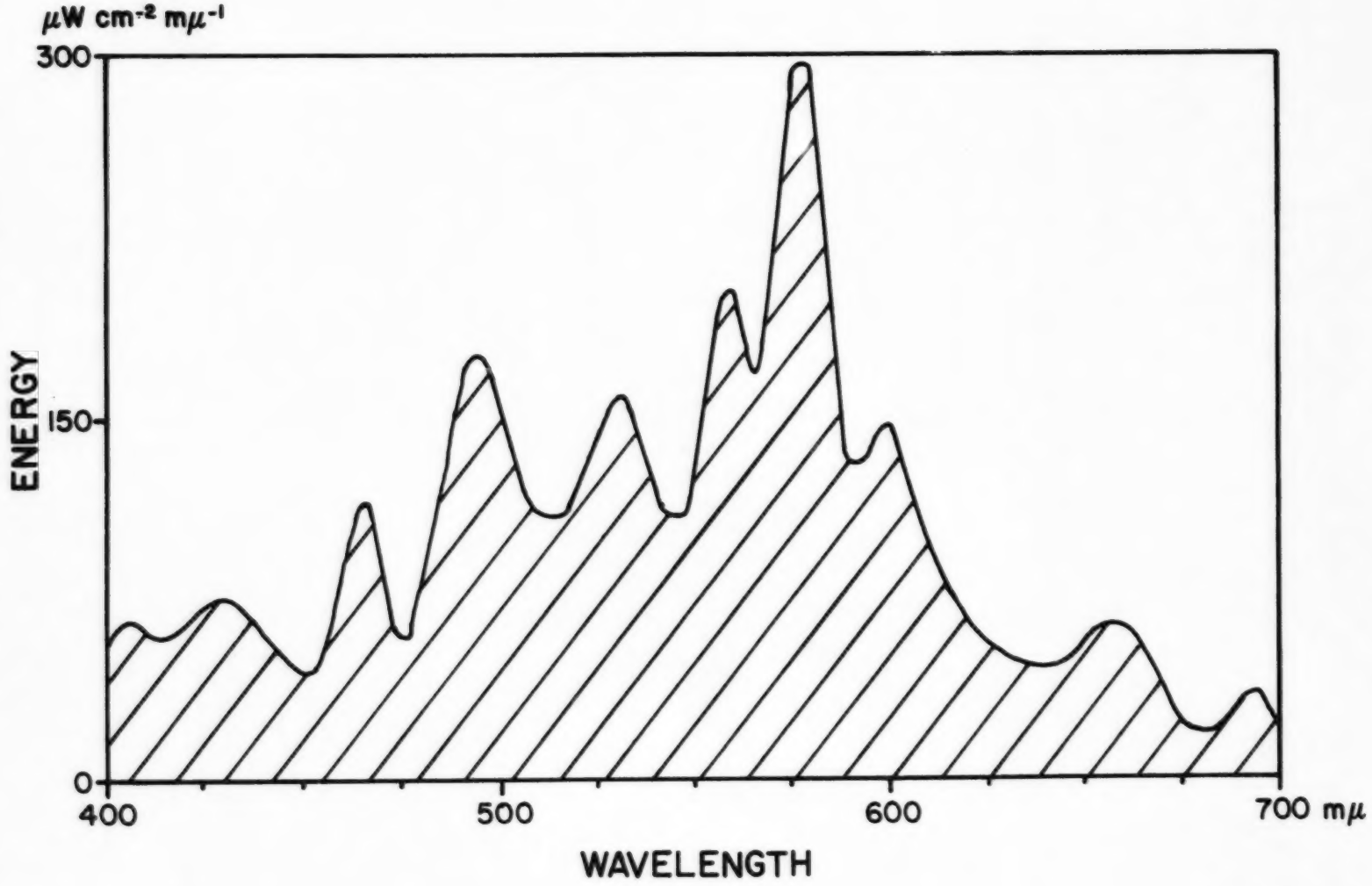


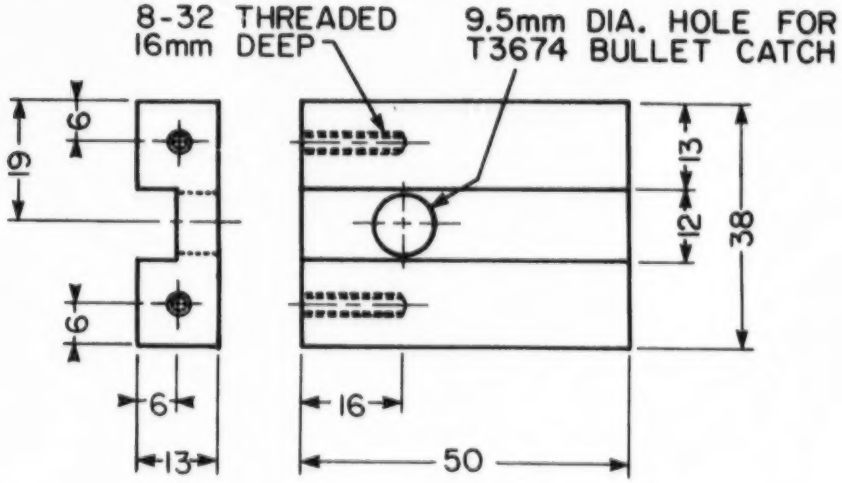
Fig. AP.3: Spectrum measured at front sample wheel of incubator (400 W coated metal halide lamp).

The flow-through water cooling system consists of a small circulating pump, a flow-through cooling coil and a 3000 $BTU \cdot h^{-1}$ compressor-condenser unit. The system has a "no flow" shutoff switch to guard against freeze up and the cooling unit is thermostatically controlled to provide temperatures from 1°C to ambient (±1°C).

The incubator light regime is usually structured to provide a 3x to 5x intensity change between any two adjacent sample wheel planes. The current design provides five sample wheels. If desired, the wheel farthest from the lamp can be made opaque and fitted with "dark bottles" to measure uptake.

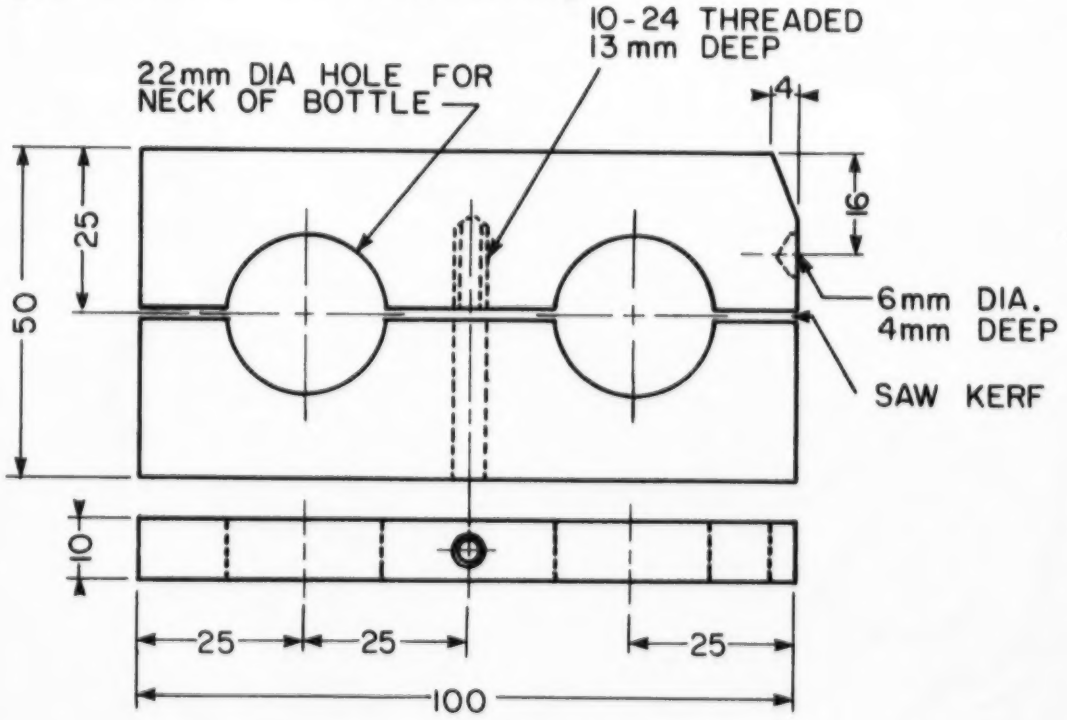
Our dark bottles are 60 mL Pyrex® reagent bottles coated with black vinyl (Cole-Parmer Plasti-dip®). Both bottles and stoppers are coated and two or three coats provide a smooth, rugged, light-tight covering.

The incubation bottles are fastened to the rotating wheels using a clamp and post arrangement (Fig. AP.4). Paired bottles are clamped together by means of the "double yoke" holder in Fig. AP.4b. Each wheel has six pairs of posts (Fig. AP.4a) spaced such that the bottle holder will slip into the slots in the posts and be held in place by a spring-loaded brass "bullet" door catch (Amerock® T-3674). The bottles are thus positioned (Fig. AP.2b) radially on the wheel with their bases pointed toward the centre.



a. BOTTLE HOLDER POST - 12 REQ'D PER WHEEL

[DIMENSIONS ARE IN MILLIMETERS]



b. BOTTLE HOLDER - 6 REQ'D PER WHEEL

Fig. AP.4: Attachment of bottles to wheels.

APPENDIX 2

"BASIC" LANGUAGE PROGRAM FOR DAILY INTEGRAL PRODUCTION CALCULATIONS

A. "BASIC" PROGRAM FOR DAILY INTEGRALS

```
1000 REM DAILY INTEGRAL PRIMARY PRODUCTION PROGRAM   EVERETT J. FEE FEB.1983
1010 REM
1020 REM THE PORTION OF THE PROGRAM THAT GENERATES A CLOUDLESS CURVE OF DAILY
1030 REM SOLAR RADIATION APPLIES TO WINNIPEG, MANITOBA AND MUST BE REWRITTEN
1040 REM FOR OTHER LATITUDES
1050 REM *NOTE* BE SURE THAT ALL INPUT DATA AND PARAMETERS HAVE THE SAME UNITS
1060 REM (METERS,MINUTES,MILLIGRAMS, AND MILLIEINSTEINS, FOR EXAMPLE)
1070 REM
1080 DIM T(31),Y(31),Q(7,18),L(8,41),P(8,41),Z(41),I(41),S(250)
1090 REM     ************************************************************
1100 REM     *THIS IS THE START OF THE PARAMETER INITIALIZATION SECTION*
1110 REM
1120 REM NC IS THE NUMBER OF CHAMBERS IN THE INCUBATOR; MAXIMUM IS 8
1130 NC=4
1140 REM I4 IS THE VALUE OF IRRADIANCE BELOW WHICH PRODN IS ASSUMED TO BE ZERO
1150 REM UNITS OF LIGHT USED HERE ARE MILLIEINSTEINS/(SQ.M. MIN)
1160 I4=.2
1170 REM N1 IS THE NUMBER OF DEPTHS AT WHICH PRODUCTION WILL BE CALCULATED;
1180 REM  THE PROGRAM CHECKS TO MAKE SURE THAT THIS NUMBER IS ODD
1190 N1=N1+11
1200 IF INT(N1/2)*2<>N1 THEN 1220
1210    N1=N1+1
1220 REM DT IS THE TIME INTERVAL BETWEEN SURFACE LIGHT DATA; UNITS USED
1230 REM  HERE ARE MINUTES
1240 DT=30
1250 REM            ***********************************************
1260 REM            *THIS IS THE START OF THE DATA INPUT SECTION*
1270 REM
1280 REM Z1 IS THE MAXIMUM DEPTH (IN METERS) TO WHICH PRODN WILL BE COMPUTED
1290 INPUT " MAXIMUM DEPTH";Z1
1300 REM INPUT NEGATIVE VALUES TO TERMINATE DATA ENTRY FOR INCUBATOR AND
1310 REM  TRANSPARENCY DATA
1320 J=1
1330 REM INPUT THE INCUBATOR IRRADIANCES AND PRIMARY PRODUCTION RATES
```

```
1340 INPUT " DEPTH RANGE (METERS) TO WHICH THESE DATA APPLY";Q(J,1),Q(J,2)
1350 IF Q(J,1) < 0 THEN 1430
1360    PRINT "INPUT INCUBATOR DATA GOING FROM LOW TO HIGH IRRADIANCES"
1370    FOR K=1 TO NC
1380        L=2*(k-1)
1390        INPUT "  IRRADIANCE, PRODUCTION(IRRAD)";Q(J,L+4),Q(J,L+3)
1400        NEXT K
1410    J=J+1
1420    GOTO 1340
1430 REM BRANCH HERE WHEN ALL INCUBATOR DATA HAVE BEEN INPUT
1440 J2=J+1
1450 J=1
1460 INPUT "  DEPTH, LIGHT(DEPTH)";Y(J),T(J)
1470 IF Y(J) < 0 THEN 1500
1480    J=J+1
1490    GOTO 1460
1500 REM BRANCH HERE WHEN ALL TRANSPARENCY DATA HAVE BEEN INPUT
1510 J1=J-1
1520 REM    ********************************************************************
1530 REM     *THE NEXT SECTION INTERPOLATES TRANSPARENCY AND INCUBATOR DATA*
1540 REM        *FOR THE DEPTHS AT WHICH CALCULATIONS ARE TO BE MADE*
1550 REM
1560 REM CONVERT TRANSPARENCY DATA TO LOGARITHMS
1570 T1=T(1)
1580 FOR K1=1 to J1
1590    T(K1)=LOG(100*T(K1)/T1)/2.30259-2
1600    NEXT K1
1610 K1=1
1620 K2=0
1630 FOR K=1 TO N1
1640     Z(K)=(K-1)*Z1/(N1-1)
1650     IF Z(K) <=1 Y(J1) THEN 1720
1660         IF K2=1 THEN 1700
1670             K2=1
1680             S1=(T(J1)-T(J1-1))/(Y(J1)-Y(J1-1))
1690             I1=T(J1-1)-S1*Y(J1-1)
```

```
1700          I(K)=S1+Z(K)+I1
1710          GOTO 1800
1720      IF Z(K)>Y(K1) THEN 1750
1730          I(K)=T(K1)
1740          GOTO 1800
1750      IF Z(K)<Y(K1+1) THEN 1790
1760          K1=K1+1
1770          IF K1 <= J1 THEN 1650
1780          GOTO 1660
1790      I(K)=T(K1+1)-(Y(K1+1)-Z(K))*(T(K1+1)-T(K1))/(Y(K1+1)-Y(K1))
1800      J4=1
1810      IF Z(K) <= Q(J4,2) THEN 1860
1820          J=J4+1
1830          IF J4 <= J2 THEN 1810
1840          PRINT "NOT ENOUGH INCUBATOR DATA";
1850          STOP
1860      FOR J5=1 TO 2*NC-1 STEP 2
1870          P((J5+1)/2,K)=Q(J4,J5+2)
1880          L((J5+1)/2,K)=Q(J4,J5+3)
1890          NEXT J5
1900      I(K)=10^I(K)
1910      C(K)=0
1920      NEXT K
1930 REM      ************************************************************
1940 REM      *THE NEXT SECTION GENERATES CLOUDLESS SOLAR RADIATION DATA*
1950 REM
1960 REM THE FORMULAS MUST BE REWRITTEN FOR EACH GEOGRAPHICAL LOCATION;
1970 REM THIS IS BEST DONE BY FITTING A COSINE CURVE TO EMPIRICAL DATA
1980 INPUT " DAY OF THE YEAR";D1
1990 S(1)=0
2000 A=1+COS(.0172142*(D1-173))
2010 L1=(485+248*A)*.959+45
2020 I1=(51.26+39.37*A)/2
2030 N-INT(L1/DT)+1
2040 L1=N*DT
2050 A1=L1/2
```

```
2060 B=10.5*3.14159/(6*L1)
2070 A2=0
2080 FOR J-2 TO N
2090    A2=A2+DT
2100    S(J)=I1*(1+COS(B*(A2-A1)))
2110    NEXT J
2120 N-N+1
2130 S(N)=0
2140 GOTO 2210
2150    REM REWRITE PROGRAM TO BRANCH HERE IF SURFACE LIGHT DATA ARE ON
2160    REM TAPE OR DISK FILE
2170    OPEN "I",#1,"SOLAR.DAT";
2180    INPUT #1,S
2190    REM R(0) MUST CONTAIN THE NUMBER OF SURFACE LIGHT DATA POINTS
2200    N=S(0)
2210 REM MAKE SURE THAN N IS AN ODD NUMBER
2220 IF INT(N/2)*2<>N THEN 2250
2230 N=N+1
2240 S(N)=0
2250 REM ***********************************************************
2260 REM *THIS IS THE START OF THE CALCULATION OF THE PRODN PROFILE*
2270 EM      * F IS THE INTEGRATION FACTOR FOR SIMPSON"S RULE *
2280 J=1
2290 F=1
2300 GOSUB 2590
2310 FOR K=2 TO N-3 STEP 2
2320    J-K
2330    F=4
2340    GOSUB 2590
2350    J=K+1
2360    F=2
2370    GOSUB 2590
2380    NEXT K
2390 J=N-1
2400 F=4
2410 GOSUB 2590
```

```
2420 J=N
2430 F=1
2440 GOSUB 2590
2450 REM PRINT THE PRIMARY PRODUCTION PROFILE
2460 PRINT "   DEPTH    TRANSP    PRODN"
2470 FOR N=1 TO N1
2480    C(N)=C(N)*DT/3
2490    PRINT USING "#####.## #####.##% #####.##";Z(N),100*I(N),C(N)
2500 NEXT N
2510 REM INTEGRATE THE VERTICAL PROFILE OF PHOTOSYNTHESIS
2520 P1=C(1)+C(N1)+4*C(N1-1)
2530 FOR J=2 TO N1-3 STEP 2
2540    P1=P1+4*C(J)+2*C(J+1)
2550    NEXT J
2560 PRINT USING "DAILY INTEGRAL PRODUCTION = #####.#";(P1*Z1/(3*(N1-1)))
2570 GOTO 2870
2580 REM       ******************************************************
2590 REM       *THIS SUBROUTINE CALCULATES THE PRODUCTION PROFILE*
2600 FOR K1=1 TO N1
2610     L1=S(J)*I(K1)
2620     REM IF LIGHT IS LESS THAN CUTOFF LEVEL THEN RETURN
2630     IF L1>14 THEN 2650
2640         RETURN
2650     IF L1>L(1,K1) THEN 2700
2660         REM INTERPOLATE LINEARLY BETWEEN 0 and PRODUCTION AT THE
2670         REM LOWEST LIGHT LEVEL IN THE INCUBATOR.
2680         P1=L1*P(1,K1)/L(1,K1)
2690         GOTO 2830
2700     IF L1 <= L(NC,K1) THEN 2750
2710         REM SET PRODN TO THAT MEASURED AT THE HIGHEST IRRADIANCE LEVEL
2720         REM  IN THE INCUBATOR.
2730         P1=P(NC,K1)
2740         GOTO 2830
2750     REM FIND THE INCUBATOR COMPARTMENTS THAT BRACKET THE VALUE OF LIGHT
2760     M=2
2770     IF L1 <= L(M,K1) THEN 2800
```

```
2780              M=M+1
2790              GOTO 2770
2800              A=P(M,K1)-P(M-1,K1)
2810              REM INTERPOLATE PRODUCTION
2820              P1-(A/(L(M,K1)-L(M-1,K1)))*(L1-L(M,K1))+P(M,K1)
2830      REM UPDATE THE PROFILE, MULTIPLYING PRODUCTION BY INTEGRATION FACTOR
2840      C(K1)=C(K1)+F*P1
2850      NEXT K1
2860 RETURN
2870 END
```

B. SAMPLE OUTPUT FROM "BASIC" PROGRAM

```
 MAXIMUM DEPTH 10
 DEPTH RANGE (METERS) TO WHICH THESE DATA APPLY 0        10
INPUT INCUBATOR DATA GOING FROM LOW TO HIGH IRRADIANCES
 IRRADIANCE, PRODUCTION (IRRAD) 1            .01
 IRRADIANCE, PRODUCTION (IRRAD) 2            .05
 IRRADIANCE, PRODUCTION (IRRAD) 8            .1
 IRRADIANCE, PRODUCTION (IRRAD) 40           .15
DEPTH RANGE (METERS) TO WHICH THESE DATA APPLY-1        -1
 DEPTH, LIGHT(DEPTH) 0        100
 DEPTH, LIGHT(DEPTH) 2         25
 DEPTH, LIGHT(DEPTH) 4         10
 DEPTH, LIGHT(DEPTH)-1         -1
DAY OF THE YEAR 290
  DEPTH     TRANSP     PRODN
   0.00    100.00%     86.90
   1.00     50.00%     74.97
   2.00     25.00%     60.30
   3.00     15.81%     51.04
   4.00     10.00%     41.03
   5.00      6.81%     31.82
   6.00      4.64%     24.42
   7.00      3.16%     17.83
   8.00      2.15%      9.44
   9.00      1.47%      4.19
  10.00      1.00%      2.65
 DAILY INTEGRAL PRODUCTION =    359.8
```

APPENDIX 3

LISTING OF DATA REPORTS

RELATING TO THIS METHODOLOGY

PRODUCTION REPORT REFERENCES

DEBRUYN, E.R., and J.A. SHEARER. 1981. Phytoplankton primary production, chlorophyll and suspended carbon in the Experimental Lakes Area - 1980 data. Can. Data Rep. Fish. Aquat. Sci. 260: iv + 52 p.

DEBRUYN, E.R., J.A. SHEARER, and D.L. FINDLAY. 1982. Phytoplankton primary production, chlorophyll, biomass and suspended carbon in the Experimental Lakes Area - 1981 data. Can. Data Rep. Fish. Aquat. Sci. 336: iv + 72 p.

DEBRUYN, E.R., J.A. SHEARER, and D.L. FINDLAY. 1984. Phytoplankton primary production, chlorophyll, biomass and suspended carbon in the Experimental Lakes Area - 1982 data. Can. Data Rep. Fish. Aquat. Sci. 438: iv + 110 p.

DECLERCQ, D.R., and J.A. SHEARER. 1976. Phytoplankton primary production in the Experimental Lakes Area using an incubator technique - 1975 data. Can. Fish. Mar. Serv. Data Rep. 647: iv + 127 p.

DECLERCQ, D.R., and J.A. SHEARER. 1978. Phytoplankton primary production, chlorophyll, and suspended carbon in the Experimental Lakes Area - 1977 data. Can. Fish. Mar. Serv. Data Rep. 74: iv + 62 p.

DECLERCQ, D.R., and J.A. SHEARER. 1979. Phytoplankton primary production, chlorophyll, and suspended carbon in the Experimental Lakes Area - 1978 data. Can. Fish. Mar. Serv. Data Rep. 137: iv + 69 p.

DECLERCQ, D.R., and J.A. SHEARER. 1980. Phytoplankton primary production, chlorophyll, and suspended carbon in the Experimental Lakes Area - 1979 data. Can. Data Rep. Fish. Aquat. Sci. 200: iv + 46 p.

DECLERCQ, D.R., J.A. SHEARER, S.L. SCHIFF, and E.J. FEE. 1977. Primary production, respiration, chlorophyll, and suspended carbon in the Experimental Lakes Area - 1976 data. Can. Fish. Mar. Serv. Data Rep. 32: v + 94 p.

FEE, E.J., D. HAYWARD, and J.A. SHEARER. 1982. Annual primary production in lakes of the Experimental Lakes Area, northwestern Ontario; 1976-1980 results. Can. Data. Rep. Fish. Aquat. Sci. 327: iv + 33 p.

SHEARER, J.A. 1976. Phytoplankton primary production in the Experimental Lakes Area using an incubator technique - 1974 data. Can. Fish. Mar. Serv. Tech. Rep. 616: v + 142 p.

SHEARER, J.A., and E.J. FEE. 1974. Phytoplankton primary production in the Experimental Lakes Area using an incubator technique - 1973 data. Can. Fish. Mar. Serv. Tech. Rep. 474: iii + 110 p.

LIGHT REPORT REFERENCES

SHEARER, J.A. 1976. Light extinction measurements in the Experimental Lakes Area - 1974 data. Can. Fish. Mar. Serv. Tech. Rep. 615: 97 p.

SHEARER, J.A., and E.R. DEBRUYN. 1981. Light attenuation in the Experimental Lakes Area - 1980 data. Can. Data Rep. Fish. Aquat. Sci. 259: iv + 50 p.

SHEARER, J.A., and E.R. DEBRUYN. 1982. Light attenuation in the Experimental Lakes Area - 1981 data. Can. Data Rep. Fish. Aquat. Sci. 337: iv + 51 p.

SHEARER, J.A., and E.R. DEBRUYN. 1983. Light attenuation in the Experimental Lakes Area - 1982 data. Can. Data Rep. Fish. Aquat. Sci. 411: iv + 63 p.

SHEARER, J.A., and D.R. DECLERCQ. 1976. Light extinction measurements in the Experimental Lakes Area. 1975 data. Can. Fish. Mar. Serv. Tech. Rep. 646: v + 113 p.

SHEARER, J.A., and D.R. DECLERCQ. 1977. Light extinction measurements in the Experimental Lakes Area - 1976 data. Can. Fish. Mar. Serv. Data Rep. 33: iv + 103 p.

SHEARER, J.A., and D.R. DECLERCQ. 1978. Light extinction measurements in the Experimental Lakes Area - 1977 data. Can. Fish. Mar. Serv. Data Rep. 73: iv + 49 p.

SHEARER, J.A., and D.R. DECLERCQ. 1979. Light extinction measurements in the Experimental Lakes Area - 1978 data. Can. Fish. Mar. Serv. Data Rep. 121: iv + 59 p.

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